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SEROEPIDEMIOLOGICAL SURVEY OF SELECTED ARBOVIRUSES IN HUMAN AND CATTLE IN GAMBELLA REGIONAL STATE, SOUTH WESTERN ETHIOPIA

PhD Dissertation

By

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Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Microbiology, Immunology and Veterinary Public Health

PhD program in Veterinary Public Health

August, 2021

Bishoftu, Ethiopia

SEROEPIDEMIOLOGICAL SURVEY OF SELECTED ARBOVIRUSES IN HUMAN AND CATTLE IN GAMBELLA REGIONAL STATE, SOUTH WESTERN ETHIOPIA

A dissertation submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Veterinary Public Health

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Title: S**eroepidemiological Survey of Selected Arboviruses in humans and cattle in Gambella Regional State, South Western Ethiopia**

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We, the members of the Examining Board of the final PhD open defense have read and evaluated the Dissertation prepared by Getahun Asebe titled: Seroepidemiological survey of Selected Arboviruses in humans and cattle in Gambella Regional State, South-Western Ethiopia, and recommend that as a partial fulfilling of the dissertation requirement for the degree of Philosophy in Veterinary Public Health.

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I hereby certify that I have read the revised version of this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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Department chair (title and name)	Signature	Date

SEROEPIDEMIOLOGICAL SURVEY OF SELECTED ARBOVIRUSES IN HUMANS AND CATTLE IN GAMBELLA REGIONAL STATE, SOUTH WESTERN ETHIOPIA

Getahun Asebe Gulich

PhD dissertation

Addis Ababa University, 2021

ABSTRACT

In the past half a century, the emergence and re-emergence of arboviral diseases have occurred in many parts of the world challenging animal and human health. Some of the arboviruses menacing the public and animal health include Zika virus (ZIKV), Yellow fever virus (YFV), Dengue fever virus (DFV), Chikungunya virus (CHIKV), Rift Valley fever virus (RVFV), and West Nile virus (WNV) in many parts of the world. Ethiopia shares borders with South Sudan, Sudan, Somalia, and Kenya, where most of the above-mentioned arboviral diseases are known to be endemic. The free movement of animals and humans across the borders potentially increases the spread of these diseases. Gambella Regional States, the current study location, shares a long border area with South Sudan, which increases the importation of diseases with livestock, wild animals, and the transhumance that travel across different countries up to Gambella. Therefore, the objectives of the study were 1)To estimate the seroprevalence of RVFV and WNV in cattle in Lare district, 2) To estimate the seroprevalence of YFV, ZIKV, and CHIKV in humans in two districts (Lare and Itang special districts) and; 3) To assess the awareness of the community about arboviruses in selected districts (Lare and Itang special districts) of Gambella Regional state, south west Ethiopia.

An indirect Enzyme-Linked Immuno Sorbent Assay (ELISA) for YFV, CHIKV, WNV, and RVFV and Blockade-of-binding ELISA for ZIKV methods were used for the seroprevalence studies. A total of 368 cattle serum samples were collected and screened for seroprevalence study of RVF and WNF from the Lare district on the border of South Sudan and measured the presence of IgG antibody against viruses' infections using enzyme-linked immunosorbent assays (ELISA). Hence, the obtained seroprevalence of anti-RVF and anti-WNF virus IgG

antibodies was 7.6% (95% CI: 5.3-10.82%), and 5.4% (95% CI: 3.52-8.29%) respectively. During the analysis, a higher seroprevalence of IgG antibodies to RVF virus infection was observed compared to the WNF virus in cattle. There was no significant association between the prevalence and the cattle age, sex, or sampled sub districts.

The community-based cross-sectional seroprevalence survey, which was conducted between late October 2018 and mid-June 2019, screened for YFV, CHIKV, and ZIKV infections. The serum samples were screened for IgG antibodies to YFV and CHIKV infections using indirect enzyme-linked immunosorbent assays (ELISA) and ZIKV infection using a specific IgG antibody with a Blockade-of-binding ELISA. Hence, a total of 150 individuals (96 males and 54 females, age ranging from 18 to 65 years, mean age ± SD = 35.92 ± 10.99 years) participated. Among the 150 samples 135, 90, and 150 were screened for YFV, CHIKV, and ZKV, respectively. The results showed that 4(2.9%), 14 (15.6%), and 41 (27.3%) samples were tested positive for IgG antibodies to YFV, CHIKV, and ZIKV infections, respectively. Agro pastoral occupation was a factor significantly associated with a high seroprevalence of IgG against CHIKV infection (AOR=14.17; 95%CI: 2.30- 87.30). Residency in the Lare district (AOR=11; 95%CI: 3.31, 39.81), being female (AOR=4.7; 95% CI: 1.62, 14.64), and pastoralist by occupation (AOR=5.1; 95% CI: 1.44-17.80) were found to be significantly associated with a high seroprevalence of IgG antibody to ZIKV infection.

A community and health facility-based qualitative study involving 11 focus group discussions (FGDs) with community members and two FGDs with health workers was conducted from November 2017 to January 2018. A total of 122 community members and 16 health workers participated in the arbovirus community awareness study. In this study, all the discussants mentioned malaria, typhoid fever, unknown cause of diarrhea, and skin diseases as the major public health problems in the area. Using pictures of *Anopheles* and *Aedes* mosquitoes, all participants confirmed that both mosquitoes are present in the area. They identified *Anopheles* as the vector of malaria. However, community discussants could not mention the name of a disease that can be transmitted by *Aedes* mosquitoes though they mentioned that *Aedes* mosquito bites both humans and animals during daytime in forest areas and causes skin itching to humans. On the other hand, community participants from the Pagag, a village bordering South Sudan, expressed a concern that *Aedes* mosquitoes can

cause a malaria-like disease, which can kill within a few days. Health workers from Itang health center described that in 2016, an outbreak of unknown disease which causes fever and jaundice occurred and killed seven individuals in a village called Akula, which is closer to a South Sudan refugee camp.

In conclusion, the findings showed that community members and health workers in the area do not have adequate information on mosquito-borne viral diseases. On the other hand, the detection of IgG antibodies to RVF, WNF, YFV, CHIKV, and ZIKV virus infections in the Gambella region warrants further study of active case findings and the dynamics of transmission. Creating awareness, improving laboratory services, strengthening the surveillance system would be important to design appropriate public and veterinary health interventions to reduce the risk of future outbreaks, early warning and preparedness in the present study area would be important.

Keywords: *Arboviruses, seroprevalence, community awareness, Gambella, Ethiopia*

STATEMENT OF AUTHOR

This thesis is solely prepared during the accomplishment of the PhD degree where all sources of material used in this thesis have been duly acknowledged. As partial fulfillment of the PhD degree, this dissertation is submitted to Addis Ababa University, College of Veterinary Medicine and Agriculture, and is deposited in the college library to be made available to borrowers under the rules of the Library. In this thesis, there is no part, which has been submitted to obtain a degree, diploma, or certificate in my name in any institution.

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College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: _____

DEDICATION

This dissertation manuscript is dedicated to my elder brother Alemu Mitiku, sister-in-law, W/r Wubayehu Tarekegn, and brother Belay Girma, for nursing me with affection and love and for their dedicated partnership in the journey of my academic life.

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LIST OF PUBLICATION

This dissertation is prepared in three parts where originally stemmed from the dissertation. The first part is published and located at the end of the document while the second and third are under considerations for publications

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Papers under consideration of publication

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ABBREVIATIONS

ANA	Antibody neutralization assay
BoFED	Bureau of Fiance and Economics Development
BSL	Biosafety level
CD	cluster of differentiation
CDC	Center for Disease Control and Prevention
cDNA	Complementary Deoxyribonucleic Acid
CHIKV	Chikungunya Virus
CNS	Central Nervous System
CSA	Central Statistics Authority
CSF	Cerebro Spinal Fluid
DFV	Dengue Fever Virus
ELISA	Enzyme Linked Immuno Sorbbent Assay
EUA	Emergency Use Authorization
FGD	Focal Group discussion
IFA	Indirect Immunofluorescence Assay
IFN	Interferon
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
m.a.s.l	Meters Above Sea Level
mAb	Monovlonal Antibody
MAC-ELISA	IgM (Immunoglobulin M) antibody capture Enzyme Linked Immuno Sorbbent Assay
MM	Milli Meter
MNA	Microneutralization assay
NAT	Nucleic Acid Testing
NS	Non-structural
OIE	Office International des Epizooties
PCR	Polymerase Chain Reaction
PRNTs	plaque-reduction neutralization tests
RNA	Ribonucleic acid

RNA-NAT	Ribonucleic acid -nucleic acid test
RT-LAMP	Reverse transcription loop-mediated isothermal amplification
RT-PCR	Reverse transcription polymerase chain reaction
RVFV	Rift Valley Fever virus
SIRS	Shock and Inflammatory Response Syndrome
SNNPR	Southern Nation and Nationality Peoples Region
WHO	World Health Organization
WNF	West Nile fever
WNV	West Nile Virus
YF	Yellow fever
YFV	Yellow Fever Virus
ZIKV	Zika Virus

1. INTRODUCTION

According to the World Health Organization (WHO), arboviruses (arthropod-borne viruses) are defined as a group of viruses that are maintained in nature principally, or to an important extent, through the biological transmission between susceptible vertebrate hosts by hematophagous (blood-feeding) arthropods including mosquitoes, ticks, and flies (Muñoz-Jordan *et al*., 2003; WHO, 1967)**.** The life cycles of all arboviruses are maintained in a complex manner that involves at least one non-human primary vertebrate host and one primary arthropod vector (Karabatsos, 1985).

The arboviruses multiply and produce viremia in reservoir vertebrate hosts, multiply in the tissues of competent arthropod vectors, and are transmitted through saliva to a new vertebrate host by the bites of arthropods after a period of the extrinsic incubation period (Davis *et al*., 2008; Peiris and Amerasinghe, 1994; Weaver and Reisen, 2010a).

The commonly known vectors for arboviruses are mosquitoes, ticks, and sandflies (Depaquit *et al*., 2012). There are also other arthropods that consume the blood of vertebrates for nutritious or developmental purposes (CDC, 2020e). Vertebrates, which have their blood, consumed act as the hosts, with each vector generally having an affinity for the blood of specific species, making those species the hosts (Kuno and Chang, 2005). Humans and other mammals affected in this cycle are known as "dead-end or incidental hosts". This means they do not develop high levels of the virus in their bloodstream needed to pass the virus to other biting mosquitoes.

In most cases, humans and domestic animals can develop clinical signs but most frequently they are 'dead-end' hosts because unable to produce significant viremia, and consequently do not contribute to the natural transmission cycle (Dobler, 1996; Gregory *et al*., 2017; Gubler, 2001; Hunt, 2015). Few arboviruses that produce viremia in people are Dengue fever virus (DFV), yellow fever virus (YFV), Rift Valley Fever virus (RVFV), chikungunya virus (CHIKV), and Zika virus (ZIKV), can be transmitted from person to person by mosquitoes (urban cycle) (Kuniholm *et al.*, 2006; Weaver and Barrett, 2004). Often these viruses have been identified with non-human primates and some domestic animals. Since early times till now over 500 different arboviruses are cataloged (Hayes *et al*., 2008; Karabatsos, 1985; van

Regenmortel *et al*., 2010). Out of these, some 100 are known to cause human disease, with clinical signs such as acute self-limiting fever (with or without rash), muscle and joint pain, hemorrhagic symptoms, and/or neurological illness. They are somehow sited specific, where a subset of these involves the central nervous system (CNS) and causing symptoms ranging in severity from mild aseptic meningitis to encephalitis or flaccid paralysis. On the other hand, most human arboviral infections are clinically weak or result in an asymptomatic infection associated with seroconversion. Fever is the most common sign and symptom of arboviral infection. Importantly the arboviruses are becoming the most significant public and veterinary health problems with the emergence and re-emergence of nearly worldwide (Weaver and Reisen, 2010a).

The growing impact of arboviruses demands continuous surveillance and monitoring especially in tropical and subtropical regions, where most of the low-income countries are located globally (Gould & Solomon, 2008). It is common to see arboviruses from many of the globe and both humans and animals are affected by these diseases (Johnson *et al*., 2012; Weaver and Reisen, 2010).

Among others, *Togaviridae* and *Bunyaviridae* virus families and the genus *Flavivirus* are the commonest causes of human arboviral infection (Schmaljohn & McClain, 1996) and animal pathogenic arboviruses belong to seven virus families:*Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae, Rhabdoviridae, Orthomyxoviridae, and Asfarviridae* (Hubálek *et al*., 2014). Current reports are showing that about 40% of the world's populations are at risk for arboviral infections. Some of the drivers for the emergence of arboviruses include deforestation, where significantly changes the breeding, abundance, and species composition of vectors. The changes in the breeding sites lead to the alteration in the immature stages, differences in resource demand, predation, survival, and fecundity. These changes may favor or disfavor vectors of arboviruses (Zahouli *et al*., 2017). Heavy rainfall is another triggering factor that increases the emergence of arthropod-borne viral diseases due to favoring the environment for vector multiplication, through the formation of small to large water bodies. The role of agriculture is also an initiating factor for most arthropod-borne diseases where new areas are frequently visited by human and domestic animals that easily connect the new host with the vectors, which are already connected with wild animal reservoirs. The climatic

changes (irregularity), demographics, climatic changes, ecology, land-use patterns, and increasing global travel have been linked to an upsurge in arboviral disease (Gould and Higgs, 2009; Weaver and Reisen, 2010a).

These viral diseases are linked to many factors (WHO-EMRO, 2007). Some of the locations where arboviruses were reported include the Somali region of Ethiopia (Ahmed & Salah, 2016), the South Omo zone(Endale *et al*., 2020; Mengesha Tsegaye *et al.*, 2018), northwest Ethiopia (Ferede *et al*., 2021).

2. LITERATURE REVIEW

2.1. Arboviruses

Arboviruses are RNA viruses transmitted via hematophagous or blood-feeding arthropods such as mosquitoes, ticks, sandflies, etc. Infections by these pathogens may result in severe disease of encephalitis and hemorrhagic fevers or mild form associated with transient fevers and polyarthritis. In classifications, arboviruses belong to seven families, namely, the; (a) *Togaviridae* family - alphaviruses (Alphavirus, one of 2 genera); (b) *Flaviviridae* family (*Flavivirus* genus); (c) *Bunyaviridae* family (*Bunyaviruses*, *nairo viruses*, and *Phlebovirus* genera); (d) Reoviridae family (*Orbiviruses* genus); (e) *Rhabdovirus* family (*Vesiculoviruses* genus); and the (f) *Orthomyxoviridae* family (*thogotoviruses* genus) (Condit, 2007; ICTV, 2011; Weaver and Reisen, 2010a). Amongst them, the most important families of the region of Africa and the Eastern Mediterranean region are the *Togaviridae*, *Flaviviridae*, and *Bunyaviridae* families. The *Flaviviruses* family was given more attention here, as most of its members belong to the emerging and re-emerging zoonosis despite the *Alphavirus* and *Phlebovirus* are resulting in health problems in humans and livestock.

2.2. Transmission

Arboviruses are transmitted primarily or mainly maintained between a host and vector cycles, which carries the virus and transmits the virus to another organism respectively (Sang and Dunster, 2001).

2.3. West Nile virus

2.3.1. Biology of West Nile virus

WNV is a mosquito-transmitted flavivirus, which belongs to the Japanese encephalitis serogroup of the family Flaviviridae. WNV is an enveloped virion containing a single-stranded, positive-sense RNA genome (Khromykh *et al*., 2001). Structurally the virus is an icosahedral particle with the capsid protein associating with the RNA genome to form the nucleocapsid surrounded by a lipid bilayer (Bhuvanakantham *et al*., 2009). The virus is well known to be neurotropic causing WNF and the leading arboviral cause of encephalitis worldwide in humans (Winkelmann *et al*., 2016).

2.3.2. Vector of WNV

The primary vector of WNV, *Culex* species of mosquito, was first discovered in Egypt. Hence, *Culex* species of mosquitoes appeared to be the primary vectors, that could maintain the vector cycle through feeding following subsequent transmission through biting (Taylor *et al*., 1956).

2.3.3. Global distribution of WNV

The virus was isolated from a febrile women patient in 1937 from the West Nile district of Northern Uganda (Smithburn *et al*., 1940), it becomes one of the most widespread arboviruses (Hayes *et al*., 2005; Gyure, 2009; Reiter, 2010a). Initially, the virus was isolated during the epidemiologic investigation of the YFV in the area. In the process of investigation, mice inoculation with the patient's serum enabled the isolation of a virus with physical and pathologic properties that are similar to those of two flaviviruses called *St. Louis encephalitis* virus and *Japanese B encephalitis* virus and sharing immunological relationships with these viruses. Hereafter, WNV occurred as an epidemic in Mediterranean basin in the 1950s and 1960s (Murgue *et al*., 2001) while the first recognized epidemic occurred in Israel in 1951 in the small town of Haifa where reports from young children take the highest proportion with no fatalities. Since then, many sporadic cases and outbreaks have been reported from Africa (Le Guenno *et al*., 1996; Nur *et al*., 1999). WNV is frequently reported as an endemic disease in Africa, Middle East, South Asia, and seldom sporadic cases in Europe (Hubálek and Halouzka, 1999; Tsai *et al*.,1998). Recently in Africa WNV has been reported in northern and sub-Saharan Africa (Cabre *et al*., 2006; El Rhaffouli *et al.*, 2012; Fassil *et al*., 2012) and East Africa (Faulde *et al*., 2012). Previously, a molecular study showed that an identical sequence of the virus from different continents showed the virus has a rapid route of transmission probably through the migratory birds (Lanciotti *et al*., 1999).

Indeed, the suggestion of WNV to be vector-borne goes back to several years based on the ecology and transmission studies so far done (Philip and Smadel, 1943). Through process further understanding on virus ecology, epidemiology, and clinical characteristics of WNV obtained during 1951 and 1954 outbreak occurred in Egypt (Gideononline, 2020; Murgue *et al*., 2001; Taylor *et al*., 1956) (figure 1). WNV found self-limited where higher

seroprevalence was found in older children while younger children seemed to have more symptomatic illness suggesting that WNV is mainly an infection of early childhood.

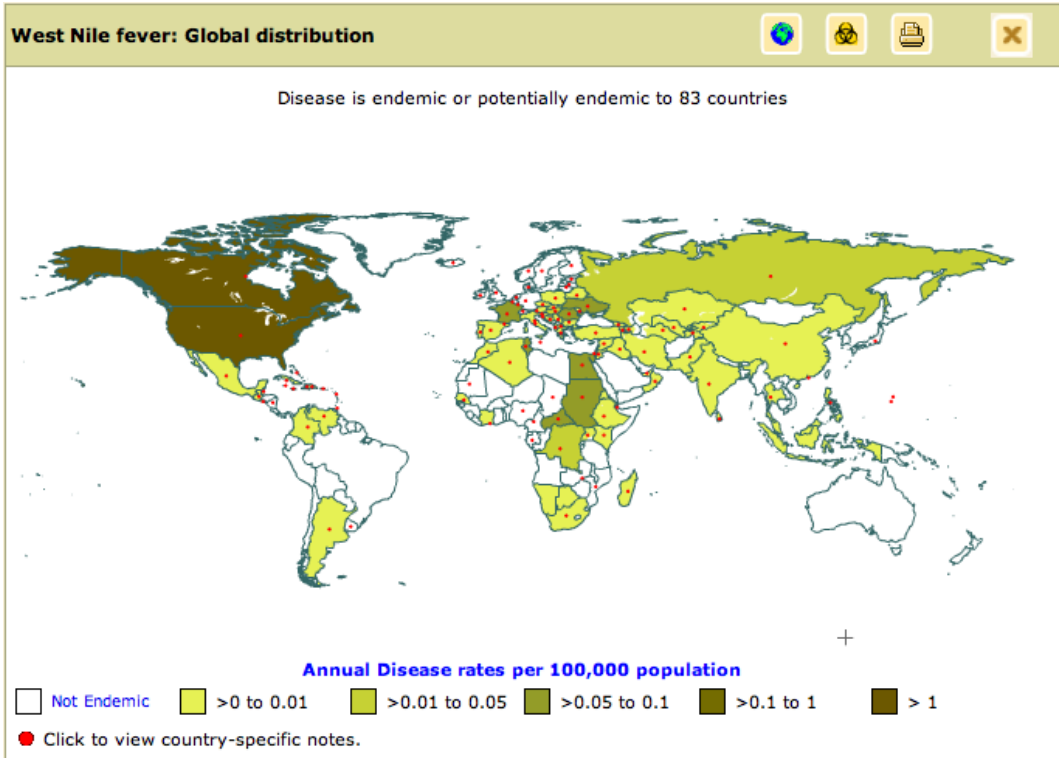


Figure 1. Global distribution of West Nile Fever.

(source: Gideononline, 2020)

2.3.3.1. West Nile virus in Ethiopia

Very limited studies have been conducted in Ethiopia regarding arboviruses infections in humans in general (Ardoin *et al.*, 1976; Woyessa *et al.*, 2013; Lilay *et al.*, 2017) despite ample evidence regarding the importance of animals and human movement between neighboring countries in the spread of arboviruses. The WNV virus was also detected from *Anopheles oustani* and *Mansonia uniformis* mosquitoes in Ethiopia (Peiris and Amerasinghe, 1994). The virus was detected in a few locations in Ethiopia (Ahmed and Salah, 2016; Hubálek and Halouzka, 1999). There is no available evidence about the status of this virus in animals in Ethiopia.

2.3.4. Life cycle and hosts of WNV

West Nile virus is mainly maintained in a life cycle between birds and the preferred vector genus *Culex* (Hubálek and Halouzka, 1999; Reiter, 2010a). In the mosquito mammalian transmission, birds play a sole role as reservoir hosts. Hereby, some species of birds become ill, show symptoms of disease, or may die: while some others become infected, serve as a carrier without showing signs of diseases. Bird-to-bird transmission has been demonstrated at the laboratory level (Komar *et al.*, 2003). In other findings, other several species have proven the capability of contact transmission (Komar *et al*., 2003). Humans and other mammals, especially horses, may develop clinical illness but usually are incidental or "dead-end" hosts because they do not produce significant viremia, and thus may not contribute to the transmission cycle (CDC, 2020g) (figure 2). In studies, a low level of virus in the blood (viremia) in mammals is mostly unable to transmit to mosquitoes upon biting, thereby ending the transmission cycle (Bowen and Nemeth, 2007). Hence, mammals' ability to act as hosts could change in such a low level of viremia phenomena, though *Aedes* mosquito biting.

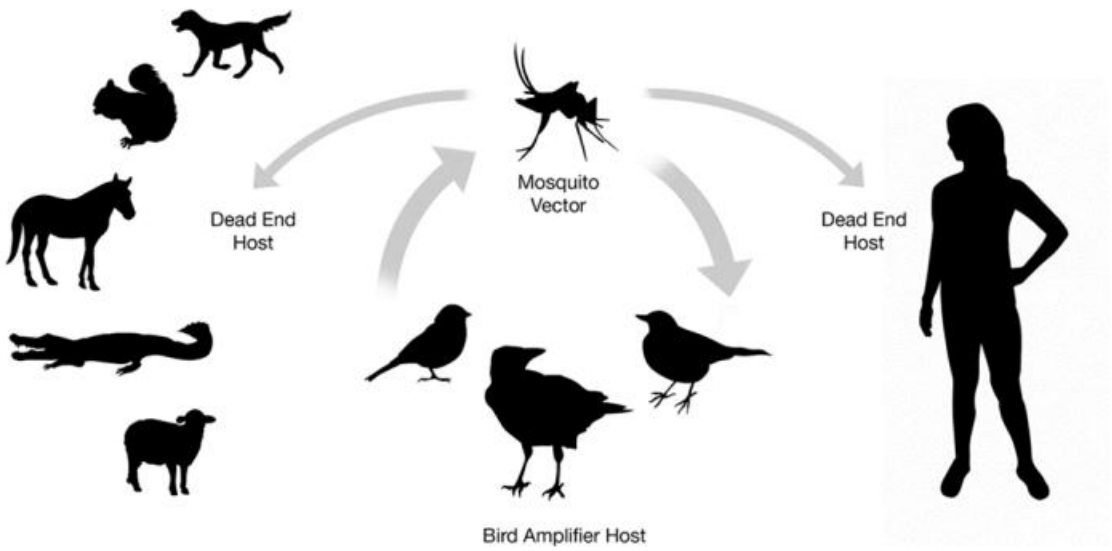


Figure 2. The life cycle of West Nile Virus

(Source (Byas & Ebel, 2020; CDC, 2020g)

2.3.5. Seroprevalence of WNV in humans

Since the first investigation of the virus from humans acts as a dead-end host, it has been being reported continuously in humans in many locations of the world (Galán-Huerta *et al*., 2015; Smithburn *et al*., 1940). WNV transmits to humans through the bite of a mosquito (Malkinson & Banet, 2002; Weaver & Reisen, 2010). Conducted studies estimated humans get an infection in a probability of one out 150 could reveal a severe neurologic symptom with a death rate of 10% among the severely affected groups(O'Leary *et al*., 2004; Petersen & Marfin, 2002). In most cases, infections persisted asymptomatic but upon revealing the infection patients might divulge headaches, myalgia, arthralgia, and rash.

2.3.6. Seroprevalence of WNV in animals

Sero-surveys investigations in animals so far suggested that the virus was found as an infectious disease in a wide range of species, including birds and non-human mammals besides human beings (Ulloa *et al*., 2003; Van Der Meulen *et al.*, 2005). The WNV-neutralizing antibodies were found prevalent in birds, and crows (Olaleye *et al*.,1990). The preferred or predominant reservoir hosts of the WNV are birds (Anderson *et al*., 1999). On the other hand, the WNV was identified and showed symptomatic cases and fatality in equines, where it continued to infect humans and /or horses nowadays too, which are dead-end hosts (Trock *et al*., 2001; Campbell *et al*., 2002; Van Der Meulen *et al*., 2005).

2.3.7. Transmission, Infection, pathogenesis and clinical manifestations in humans

As previously indicated the dominant mode of WNV transmission is through the *Culex* mosquito. Transmission via non-vector routes also reported including organ transmission (Iwamoto *et al*., 2003), blood transfusion, breastfeeding, and intrauterine exposures (Hayes and O'Leary, 2004). There is also a report of laboratory-acquired infection (Centers for Disease Control and Prevention, 2002).

WNV infection is predominantly subclinical or self-limited. Seldom reports indicated infection manifestations may range from fever and myalgias to meningoencephalitis and death in humans (Petersen and Marfin, 2002). Higher seroprevalence was seen in older children while younger children seemed to have more symptomatic illness suggesting that

WNV is mainly an infection of early childhood. In most cases, human infections with WNV remain clinically silent. Among those persons developing the asymptomatic illness, most develop a self-limited febrile illness. Severe illness with WNV is manifested as meningitis, encephalitis, or acute anterior (polio) myelitis. These manifestations are generally more prevalent in older persons or those with immunosuppression (Campbell *et al*., 2002; Sejvar, 2014).

The risk factors for WNV in humans are categorized into two as mosquito and non-mosquito related. People get WNV infection after exposure to potentially infected *Culex* spp mosquito. Many findings documented risk factors for infection with this virus including; availability of water/flooded areas (Han *et al*., 1999), presence of more vegetation cover (Brownstein *et al*., 2002), old houses, low population density, and proximity to bird populated areas as mosquito-related risky factors (Ruiz *et al.*, 2004).

The non-mosquito-related risk factors for WNV infection include; untested blood transfusions or organ transplantations (Kumar *et al*., 2004), breastfeeding, or infection during pregnancy (Hayes and O'Leary, 2004), and occupational exposure to the virus (Centers for Disease Control and Prevention, 2002).

2.3.8. Transmission, Infection, pathogenesis and clinical manifestations animals

The WNV passes to the birds through the bite of infected mosquitoes where these mosquitoes before infected during biting of birds. Species susceptibility of birds to WNV is detected as crows, jays, and sparrows are highly susceptible to WNV compared to other species of birds (Ip *et al*., 2014). The nature of predatory in hawks and owls and scavenging of crows birds increases the WNV infection easily because they have a chance to eat WNV infected or sick/dead birds (Anderson *et al*., 1999; Ip *et al*., 2014).

Clinical manifestation in birds varies based on the situation. As birds are the natural amplifiers for the WNV(Komar, 2001), observing clinical manifestations depends on factors including; species, previous virus history of the area. Hence, upon critical observation of the infected birds, it is possible to see nonspecific signs of illness including dehydration, emaciation, and depression. Other signs include neurologic abnormalities such as head

tremors, ataxia, head incoordination, torticollis, nystagmus, and head tilt. Depending on the species of birds affected it was also exhibited anemia and leukocytosis with heterophilia, eosinophilia, and monocytosis consistent with chronic inflammation (Joyner *et al*., 2006).

Risk factors for birds to be infected with WNV generally include; climate, mosquito habitats, and an immunologically young population of birds. Individual variations within the birds may also contribute to the risk of severe disease. Variation in susceptibility among different ages and sex has not shown significant changes in many studies so far conducted (Smith *et al*., 2018).

Like the human, WNV infection is often asymptomatic in equines (Campbell *et al*., 2002). The first detection of WNV in equines was noted in Egypt and France in the early 1960s (Haahr, 1969; Schmidt and El Mansoury, 1963). Equines get the WNV infection after bitten by an infected potential mosquito. In equines, the incubation period usually ranges from three weeks.

Clinical manifestations of WNV in equines may not be easy to distinguish from other equine encephalitides. Like the WNV, other causes including rabies, equine herpes virus-1, equine protozoal myeloencephalitis, and Eastern, Western, or Venezuelan equine encephalomyelitides results in encephalitides (Smith, 2002). In affected horses, clinical signs may include both central and peripheral nervous system signs, ataxia, lack of interest in surroundings, weakness of limbs, muscle fasciculation, and loss of appetite (Government of Canada, 2020).

Sometimes horses may become recumbent and may be unable to get up without help. Some horses may develop a fever. The target organ of the virus is the brain and spinal cord where it causes an inflammatory reaction (encephalitis), manifested by variable neurological signs (Abutarbush *et al*., 2004). Affected horses, which show no progression of gait abnormalities, usually recover completely with no consequences within one to two weeks. Consequently, specific diagnostic tests need to be conducted to differentiate or have a definitive diagnostic result. Samples sources, in this case, could be, either the whole blood, serum, and/or cerebrospinal fluid (CSF) with a complete history of the case is satisfactory. Depending upon a country protocol, specific tests for rabies and WNV could be done in equines when

recumbency occurred, including postmortem examination(Abutarbush *et al*., 2004; Snook *et al*., 2001).

A WNV-infected *Culex* mosquito can bite cattle as mammals where antibody was detected in few studies conducted elsewhere(Olaleye *et al*., 1990; Omilabu *et al*.,1990; Ozkul *et al*., 2006). The clinical importance of WNV in cattle has not been documented so far, despite the reports of immune response antibody detections against WNV observed (Ilkal *et al*., 1988).

2.3.9. Host immune response to WNV

After the virus enters the CNS through respective tissues, it spreads rapidly between different subtypes of neurons in distinct regions of the nervous systems. Largely, the neurons are non-renewable (McGavern and Kang, 2011). Controlled immune responses must limit spread and eliminate virus while minimizing neuronal damage, but when there is a delay or absence of such responses in genetically deficient mice or immunosuppressed humans results in rapid dissemination, neuronal injury, with an increased risk of mortality. Animal model-based research work has shown that both innate and cellular immune responses in the CNS orchestrate control of WNV spread, which ultimately limits the number of neurons targeted for infection or the amount of virus any given infected neurons will produce (Griffin, 2003).

Many of the humoral immune response analyses to WNV have mainly been carried out subsequently from experimental infection of rodents. The antigenic variability of WNV has been determined using the isolation of monoclonal antibody (mAb) panels and challenge studies have been performed to test vaccines for clinical purposes. Serological responses to the virus infection have been used in the classical observation of the human antibody response. In recent updates, many advanced protein-protein engineering approaches and display technologies have been utilized to probe the human repertoire against WNV. Collectively, these studies have identified important differences in the humoral response against natural WNV infection in humans compared to experimental WNV infection in rodents (Oliphant *et al.*, 2005; Sánchez *et al*., 2005).

2.3.10. Diagnosis of WNV

2.3.10.1. Clinical diagnosis

Suspicion of WNV infection can be expected when patients have the onset of unexplained febrile illness, encephalitis and/or meningitis, or flaccid paralysis during mosquito season. Evidence of WNV enzootic activity or other human cases, either locally or in a region where the patient has traveled, should raise the index of suspicion (Grant *et al*., 2011; Davis *et al*., 2008).

2.3.10.2. Serologic diagnostic methods of West Nile Virus

Currently, the serologic diagnostic methods of WNV include immunoglobulin M antibody-capture ELISA, immunoglobulin G ELISA, indirect fluorescent antibody tests, hemagglutination inhibition tests, and plaque reduction neutralization tests (Shi and Wong, 2014). Preferably, WNV infection is better diagnosed through the detection of IgM in serum or cerebrospinal fluid (CSF) however, because of cross-reactivity between flaviviruses, positive results need to be confirmed by virus neutralization assay.

According to the CDC guideline, WNV-specific IgM antibodies are usually detectable 3 to 8 days after onset of illness and persist for 30 to 90 days, but also longer persistence has been documented. Consequently, positive IgM antibodies seldom may reflect a past infection of the host. If serum is collected within 8 days of illness onset, the absence of detectable virus-specific IgM does not rule out the diagnosis of WNV infection, and the test may need to be repeated on a later sample. Taking into account the presence of IgM specific to WNV in blood or CSF gives good evidence of recent infection but may also result from cross-reactive antibodies after infection with other flaviviruses or from non-specific reactivity. For confirmation, neutralizing antibody testing of acute and convalescent-phase serum specimens at a state public health laboratory (CDC, 2020d). Opposite to WNV, IgM, and IgG of other viral infections can persist for many years following a symptomatic or asymptomatic infection. The presence of IgG is only evidence of the previous infection (figure 3). When capacities are available the plaque-reduction neutralization tests (PRNTs) performed in reference laboratories, can help determine the specific infecting flavivirus. PRNTs can also confirm acute infection by demonstrating a fourfold or greater change in WNV-specific

neutralizing antibody titer between acute- and convalescent-phase serum samples collected 2 to 3 weeks apart.

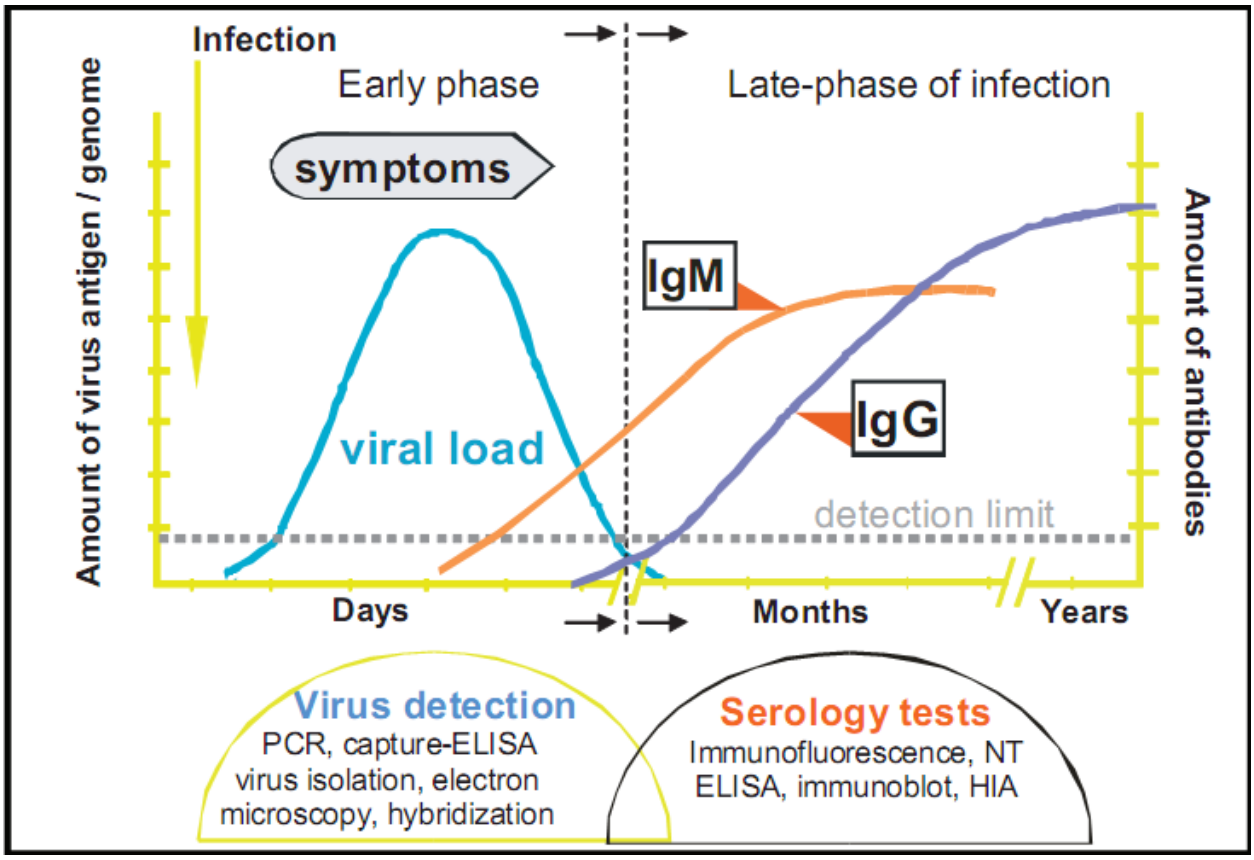


Figure 3. Schema for the diagnosis of viral infections

Source: (Donoso, 2011)

In addition to serum and viral culture systems through the immunohistochemistry (IHC) method, the WNV antigen can be detected in formalin-fixed tissue. Negative results of these tests do not rule out WNV infection.

2.3.10.3. Molecular diagnosis of West Nile virus

In many cases of WNV viral confirmation, the viral cultures and tests to detect viral RNA (e.g., reverse transcriptase-polymerase Chain Reaction [RT-PCR]) can be performed on serum, CSF, and tissue specimens that are collected early in the course of illness for positive results. Mostly, the early WNV infection can be diagnosed by PCR and virus isolation (CDC,

2020d). Viral culture, RT-PCR, and IHC can be requested through state public health laboratories or CDC for confirmatory diagnosis (Sampathkumar, 2003).

2.3.11. Control of WNV

At this time, no definitive treatment is available for WNV infection. Prevention of infection through protection from mosquito bites remained the most critical, the most important, and the single public health measure. In the absence of definitive antiviral treatment, management of illness due to WNV infection remains supportive. Patients with otherwise uncomplicated WNF generally do not require specific intervention, though control of a headache and rehydration may sometimes be needed. However, persons with documented West Nile viremia and patients with WNF in which other risk factors, including older age and underlying immunosuppression, are present should be observed for progression to more severe neuroinvasive disease (CDC, 2020d; Sejvar, 2014). Avoid blood donating before testing of West Nile virus (Brown, 2004).

2.4. Zika Virus

2.4.1. Biology of Zika Virus

Zika is among the emerging viral disease of a family *Flaviviridae* and genus *flavivirus*, which is transmitted by a daytime active mosquito called *Aedes* with the two most commonly known species as *A. aegypti*, *A. albopictus* that also may bite at night period. The name Zika came from the natural forest found in Uganda Zika forest where the virus was first isolated in 1947 (Dick *et al*., 1952). In its character, ZIKV can infect several cell types in the skin and, like many flaviviruses, ZIKV also uses multiple 'receptors' to mediate attachment and entry (Hamel *et al.*, 2015). Similar to other members of the *Flavivirus* genus, it contains a positive, single-stranded genomic RNA encoding a polyprotein that is processed into three structural proteins, i.e., the capsid (C), the precursor of the membrane (prM), and the envelope (E), and seven nonstructural proteins, i.e., NS1 to NS5 (Kuno *et al*., 1998). Virus replication occurs in the cellular cytoplasm of the host cell.

2.4.2. Global distribution of Zika virus

Literature documented that it was first discovered during scientists conducting a YF study in Uganda at the Zika forest in 1947 accidentally where they also observed a fevered rhesus macaque placed in a cage near the East African Virus Research Institute in Entebbe. The monkey that was kept for YF study purpose developed a fever, and researchers isolated from its serum a transmissible agent that was first described as ZIKV in 1952 (Dick *et al*., 1952). Arguments between many researchers showed that ZIKV has probably been circulating in nature in a sylvatic cycle for many decades and seldom spilling undetected into human populations across Africa with unknown regularity (Nutt and Adams, 2017).

Later on, following the virus isolation, the first ZIKV case was reported in the human being in Uganda and Tanzania in 1954 (Dick, 1952). Before 2007, there were rare reports of confirmed cases of ZIKV infection from Africa and the Southeast Asian continent. Later on, in 2007, a major epidemic occurred in Yap Island (Micronesia) which involved about 5000 persons (Duffy *et al*., 2009; Lanciotti *et al*., 2008) and Gabon (Grard *et al*., 2014). The outbreak on the island of Yap in the Federated States of Micronesia in April 2007 was the first to occur outside of Africa and Asia (figure 4).

The virus has spread to the South and Central America and the Caribbean area since April 2015. From January 2016, the CDC gives a frequent alert for travelers, pregnant women, and those who have the intention to be pregnant. WHO had been alerted the virus could likely spread further throughout most of America during that time (Hennessey *et al*., 2016).

Starting from early 2016, a widespread outbreak of ZIKV is actively flourishing in the Americas make the starting point as early as April 2015 from Brazil then continue to other areas of South America, Central America, Mexico, and the Caribbean (Ikejezie *et al*., 2017).

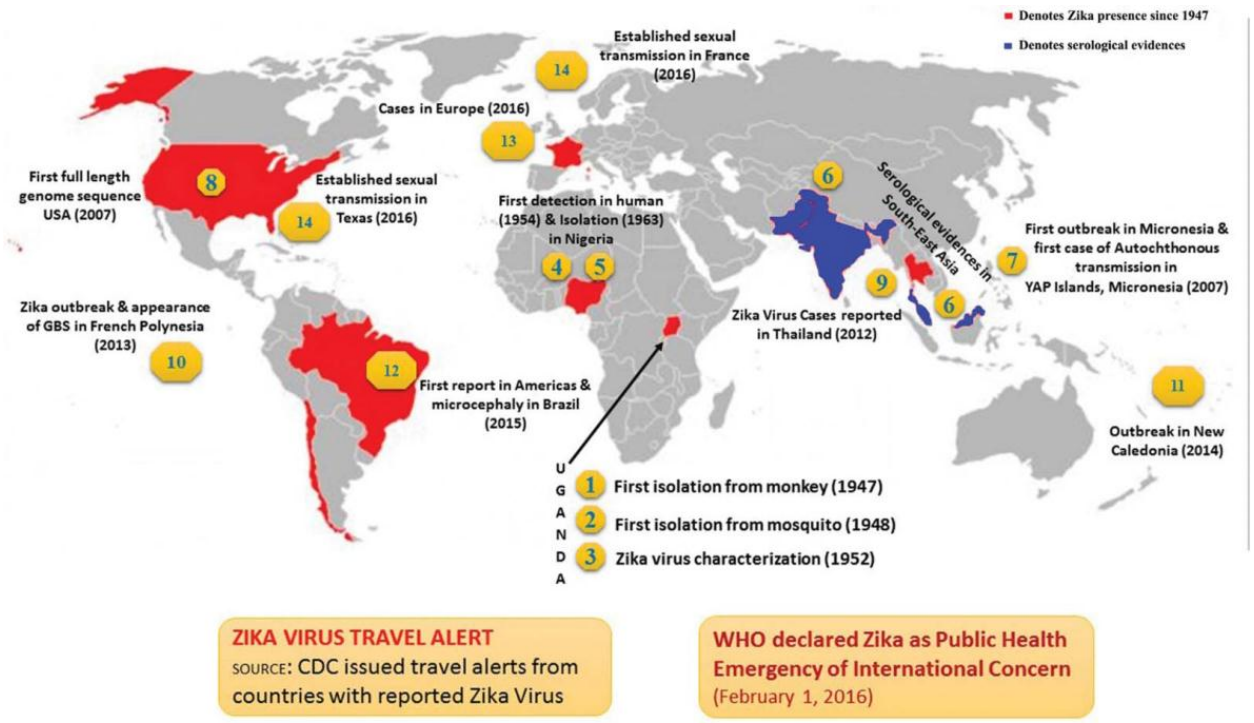


Figure 4. How Zika virus spread from Uganda in Africa (Landmarks in Zika virus epidemiology.

The red area depicts Zika virus presence and/or serological reports (Since 1947-2016) and in blue denoted serological evidence of Zika in Southeast Asia)

Source: (Singh *et al.*, 2016)

2.4.3. Zika in Ethiopia

Among the arboviruses, Zika is a poorly studied virus in Ethiopia. A study by Mengesha Tsegaye *et al.*(2018) revealed a 0.4% prevalence in two study zones of Ethiopia, that did not include the present study area (Mengesha Tsegaye *et al.*, 2018).

2.4.4. Hosts and life cycle of Zika virus

ZIKV hosts include many hosts including human beings. The virus has been detected in primates, wild and domestic animals including, monkeys, sheep, goats, horses, cows, ducks,

rodents, bats, orangutans, and carabaos (Althouse *et al*., 2015; Vorou, 2016). Bueno *et al*.(2016) recommended that much wildlife could be a reservoir and sylvatic hosts of ZIKV that demands detailed investigations. Anti-ZIKV antibodies were also detected in wild mammals in Senegal in 1967–1968(Bres, 1970; Diallo *et al.*, 2014).

Besides the vector *Aedes aegypti* mosquitoes, the virus was found to transmit through sexual intercourse. The virus-infected mosquitoes will infect a first-person where at this time the individual will acquire the virus. The first infected person will transmit the virus either sexually or being infected by a potential mosquito. The virus then circulates between a person/animal and the mosquito (Whitehead *et al*.,2007) (figure 5).

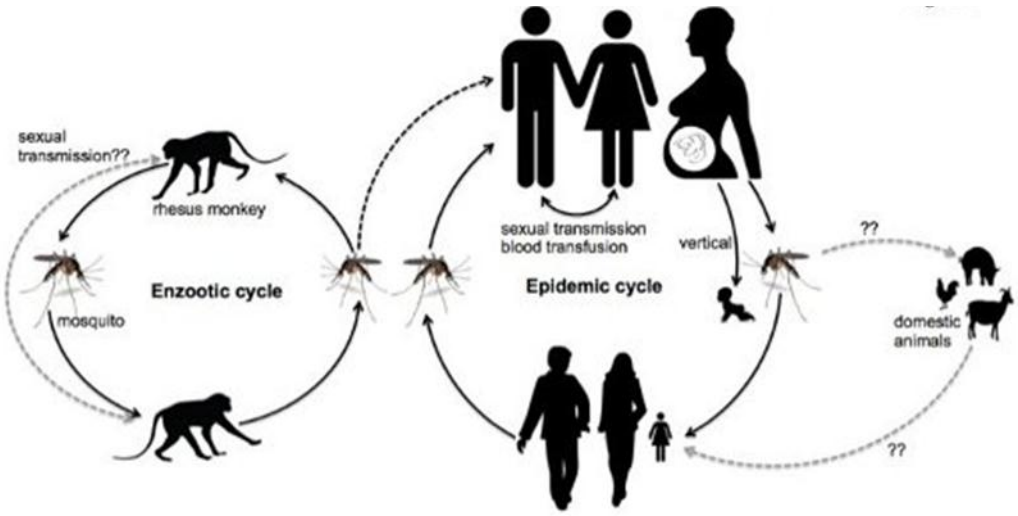


Figure 5. The life cycle of the Zika virus

(Michigan Tech, 2020)

2.4.5. seroprevalence of ZIKV in humans

Since 1952 where the first virus was first identified in humans, it has been reported in many parts of the world in humans as a primarily affected host (Dick *et al.*, 1952; Duffy *et al*., 2009; Musso *et al*., 2016).

2.4.6. seroprevalence of ZIKV in animals

Initially, the virus has been isolated from monkeys (McCrae and Kirya, 1982). The virus also has been reported in many non-human primates, wildlife, and other ungulates that are also found infected with the zika virus (Darwish *et al*., 1983; Musso *et al*., 2015). Anti-ZIKV antibodies were detected in ducks, goats, cows, horses, bats, and carabaos (water buffalo), indicating the widespread circulation of the virus in domestic animals. The question of whether birds transfer the virus over long distances remains unanswered (Olson *et al*., 1983).

2.4.7. Transmission, infection, pathogenesis and clinical manifestations in humans and animals

The virus primarily passes after the bite of an infected mosquito (*Aedes aegypti*). After mosquito bite or another route of transmission of the virus, the virus is thought to replicate initially in dendritic cells near the site of inoculation then spread to lymph nodes and the bloodstream. Even though flaviviral replication is assumed to occur in cellular cytoplasm, a study also suggested that ZIKV antigens could be found in infected cell nuclei (Buckley and Gould, 1988). Regarding the period of viral detection, it has been detected in human blood as early as the day of illness onset and viral nucleic acid has been detected as late as 11 days after onset (Filipe *et al*., 1973; Lanciotti *et al*., 2008). Viral isolation was done from the serum of a monkey 9 days later after experimental inoculation (Dick *et al*., 1952).

The illness after the Zika virus is not life-threatening and notoriously, it is serious mainly because of its link to the birth defect microcephaly where babies to be born with abnormally small heads and adults with neurological signs (Macdonald *et al*., 2014). According to the WHO notification, in the last five years, extensive studies showed the virus is spreading alarmingly. Therefore, ZIKV has been declared as an international public health emergency (WHO, 2020b) even though the virus is not life-threatening as such.

Simpson was the first person who well-documented human ZIKV diseases in 1964 after his own occupationally acquired ZIKV illness at age 28 (Simpson, 1964). It began with a mild headache followed by a maculopapular rash covering the face, neck, trunk, and upper arms, and spread to the palms and soles. Transient fever, malaise, and back pain also developed. By

the evening of the second day of illness, he was afebrile, the rash was fading, and he felt better. The virus was isolated during the febrile period.

Similarly, in 1973, Filipe *et al*. (1973) reported laboratory-acquired ZIKV illness in a man with an acute onset of fever, headache, and joint pain but no rash. ZIKV was isolated from serum collected on the first day of symptoms; the man's illness resolved in ≈1 week. Fever is the most common sign in ZIKV patients(Olson *et al*., 1981) Other manifestations included anorexia, diarrhea, constipation, abdominal pain, dizziness, conjunctivitis, and arthralgia (Duffy *et al*., 2009; Lanciotti *et al*., 2008). Other less frequent manifestations included myalgia, headache, retroorbital pain, edema, and vomiting (Duffy *et al*., 2009).

2.4.8. Transmission, infection, pathogenesis and clinical manifestations in humans and animals

In literature, so far there have not been any reports of animals becoming sick with ZIKV. Also, no confirmed report suggests animals contribute to the spread of ZIKV. Besides, there is no evidence that ZIKV spreads to people from direct contact with animals (CDC, 2018)

2.4.9. Host immune response

Similar to other infections, the early responses by the innate immune system are the first line of host defense for the suppression of viral infections. IFN production is a major component of the innate response and the transcriptional regulation of numerous IFN-regulated genes leads to an antiviral environment (Schneider *et al*., 2014). Humoral Immunity is another immune response to Zika virus infection that produces neutralizing antibodies that are thought to be a major factor in the protection against ZIKV infection (Sapparapu *et al*., 2016; Stettler *et al.*, 2016; Swanstrom *et al*., 2016). The third immune response for ZIKV infection is the cell-mediated immunity system. Studies for many flaviviruses such as dengue fever, yellow fever, and Japanese encephalitis showed that there are strong CD4+ and CD8+ T cell responses. With this fact, findings presented the argument that due to the high degree of sequence homology between DENV and ZIKV, some of the HLA-restricted CD8+ T cell epitopes may be conserved (Rivino and Lim, 2016). ZIKV-specific T cell responses have been elaborated on in animal models and humans (Stettler *et al*., 2016).

2.4.10. Diagnosis of Zika virus

2.4.10.1. Clinical diagnosis

Clinical signs of Zika virus infection are not specific and confident to confirm the virus presence. The increment of neurological syndromes such as Guillain-Barré Syndrome, other neurological syndromes (meningitis, meningoencephalitis, and myelitis), increase in microcephaly, and other congenital anomalies (Pan American Health Organization / World Health Organization, 2016). Clinical based diagnosis of the Zika virus is difficult (Ioos *et al*., 2014).

2.4.10.2. Serological diagnosis

During the serological ZIKV examination, ELISA and Indirect Immunofluorescence (IIFT) may be used for indirect determination of the presence of the antibodies of the virus(Elisa, 2016). The serologic test for Zika virus is virus-specific to IgM and neutralizing antibodies typically develop toward the end of the first week of illness. IgM levels are variable, but generally are positive starting near day four post onset of symptoms and continuing for 12 weeks. The other CDC recommended diagnostic method is the Zika MAC-ELISA method where the Zika IgM antibody capture enzyme-linked Immunosorbent Assay (Zika MAC-ELISA) is used for the qualitative detection of Zika virus IgM antibodies in serum or cerebrospinal fluid; however, due to cross-reaction with other flaviviruses and possible nonspecific reactivity, results may be difficult to interpret. Consequently, presumed positive, equivocal, or inconclusive tests must be forwarded for confirmation by plaque-reduction neutralization testing (PRNT). PRNT is performed as a CDC-designated confirmatory testing laboratory to confirm presumed positive, equivocal, or inconclusive IgM results After fever onset and viremia occurred due to the viral RNA on the course of infection, there is a development of IgG antibody following the IgA/IgM detection. In the course of infection detection, these antibody classes may be observed at the same time(Elisa, 2016). Detection of the IgG against the Zika virus is also another screening method to identify the previous infections of the hosts (Elisa, 2016; Liao *et al*., 2020). ZIKV has a high structural similarity with other flaviviruses that can cause cross-reactions of the specifically mentioned antibodies, hence serological interpretations require additional parameters. Serological interpretations should consider into account the previous history of contact with other

flaviviruses and vaccinations. In serological ZIKV examinations, using a highly specific NS1 antigen excludes the cross-reactivity issue (Elisa, 2016). Serological testing is recommended for ZIKV infections older than 7 days, although, antibodies can be detected in the blood from day five onwards.

2.4.10.3. Molecular diagnosis

Detection of Viral RNA depends on the disease stage. Hence, the molecular testing used for symptomatic persons with Zika virus infection was detected in the early course of illness. In serum diagnosis, an RNA NAT (nucleic acid testing) based testing should be performed in the first two weeks after symptom onset. Similarly, during urine sample diagnosis, an RNA NAT testing should also be conducted within less than 14 days after symptom onset. According to the CDC, a diagnosis for Zika virus includes the Trioplex Real-Time RT-PCR (rRT-PCR) assay. This method is only recommended for use under an Emergency Use Authorization (EUA) (CDC, 2019a). The Trioplex Real-Time RT-PCR (rRt-PCR) is used to detect Zika, dengue, and chikungunya RNA viruses. Variable duration after infection onset is recommended for virus detection using PCR. In a pregnant woman, the virus could be detected after several weeks in individual cases. Compare to serum or plasma, ZIKV could be detected in urine by PCR for a longer period. On the other hand, urine should always be collected with a patient-matched serum specimen. A positive RNA NAT result on any sample confirms ZIKV infection and no additional testing is indicated. A negative RNA NAT result does not exclude ZIKV infection and serum should be analyzed by IgM antibody (serological) testing. Direct pathogen detection with real-time PCR is also possible in the early phase of the virus infection. This direct detection is accomplished with the reverse transcription of the viral RNA into complementary DNA (cDNA), followed by the PCR amplification and fluorescence-based real-time detection of virus genome sequences (Elisa, 2016).

2.4.11. Control of Zika virus

WHO recommends protection of ZIKV can be done by preventing mosquitoes from bit and removing their breeding sites, avoid unsafe sexual intercourse, and planning traveling sites (CDC, 2019; WHO, 2018c). Prevention and reduction of mosquitoes could be done through

insect repellent, wearing clothes (light-colored), using physical barriers such as screens, closed doors, and windows, and sleeping under mosquito nets. Making empty or clean or cover containers that can hold water such as buckets, flower pots, or tires to reduce or make unavailable for breeding sites (CDC, 2016; WHO, 2018c).

The ZIKV disease is naturally relatively mild and requires no specific treatment. Getting plenty of rest, drinking enough fluids, and treat pain and fever with common medicines are recommended when a ZIKV infection occurred. In cases of poor recovery after going through these steps, it is better to seek medical care and advice. There is currently no vaccine available (CDC, 2016; WHO, 2018c).

2.5. Yellow Fever

2.5.1. Biology of Yellow fever

Yellow fever virus (YFV) is the model of the genus *Flavivirus* of the family *Flaviviridae* and causes YF disease (Monath and Nystrom, 1984). YFV has two biological properties, viscerotropism, and neurotropism, which are basic characteristics for wild-type YFV strains. These characteristics are associated with intense shock and inflammatory response syndrome (SIRS), likely mediated by pro-inflammatory cytokines and cytokine dysregulation (Marfin & Monath, 2008). The YFV genus consists of at least 68 viruses grouped based on shared antigenic determinants and physicochemical properties. The relatively specific neutralization test using polyclonal antisera has been used to distinguish at least eight antigenic complexes to which closely related *flaviviruses* are assigned (Monath, 1999). The virus could live commensally with the forest and bush-living mosquitoes including *Aedes* spp., *Haemagogus* spp., and *Sabethes* spp., after infecting the mosquitoes. In the case of *A. aegypti* the virus may persist through the dry season by transovarial transmission. The mosquito represents the reservoir host and largely determines the geographic distribution and persistence of the virus in nature (Abee *et al*., 2012).

2.5.2. Global distribution of yellow fever

Yellow fever is a mosquito-borne viral disease, originally came from West Africa specifically (Cliff *et al*.,2004). In the 16^{th} or 17^{th} century, the larvae of its carrier, the *Aedes*

aegypti mosquito was likely transported from West Africa to the New World in water casks carried aboard via ships carrying slaves ships and found an ecological niche in its new home (Facts and details, 2014; Pedro, 2009). It is known to cause hemorrhagic fever. The disease is endemic in equatorial Africa and South America (Gould *et al*., 1989; Monath, 2001). Specifically, the disease occurs in tropical areas of Africa and South America, and each year there are an estimated 200,000 cases of YF worldwide, leading to approximately 30,000 deaths (MedicineNet, 2020; Robertson *et al*.,1996).

Based on WHO annual reports, the occurrence of YF is mainly concentrated in 47 endemic countries in Africa in Central and South America. Of the total, 90% of cases of YF reported every year comes from Sub-Saharan Africa (Facts and details, 2014; WHO, 2017b) (Figure 7). In YF history, the first epidemics occurred in Mexico and Guadeloupe in the year 1648. Then, a regular and devastating epidemic of YF followed across the Caribbean, Central and South America, the southern United States, and Europe throughout the 18^{th} and 19^{th} centuries. The epidemic was able to cease the slave trade for a while until a formal quarantine system was established (Pearson and Miles, 1980). The quarantine system was already implemented in Europe due to the plaque that occurred before YF. In the USA urban form of the YF continued, kill the inhabitants of Philadelphia. The high mortality due to YF leads to the failure of the French Panama canal (Gallup and Sachs, 2000; McCullough, 1977). In Europe, even the natural sylvatic cycle of yellow fever is not feasible, YF transmission with infected tourists from Latin America and Africa would remain the potential source of infection for many touristic areas due to the presence and increased distribution of potential mosquitoes that transmits the virus (Javelle *et al*., 2019). The virus is reported in many African countries, mainly the sub-Saharan region. According to a WHO report, more than 27 countries are at the highest risk for YF epidemics in Africa. Overall, the estimate of YF burden is at 84,000–170,000 severe cases and 29,000–60,000 deaths annually (WHO, 2020d). Some of the countries that reported YFV in Kenya (Chepkorir *et al*., 2019), Uganda(WHO, 2011), Ethiopia (Lilay *et al*., 2017), South Sudan (Onyango *et al*., 2004), Angola, and the Democratic Republic of Congo (Kraemer *et al*., 2017) (figure 6).

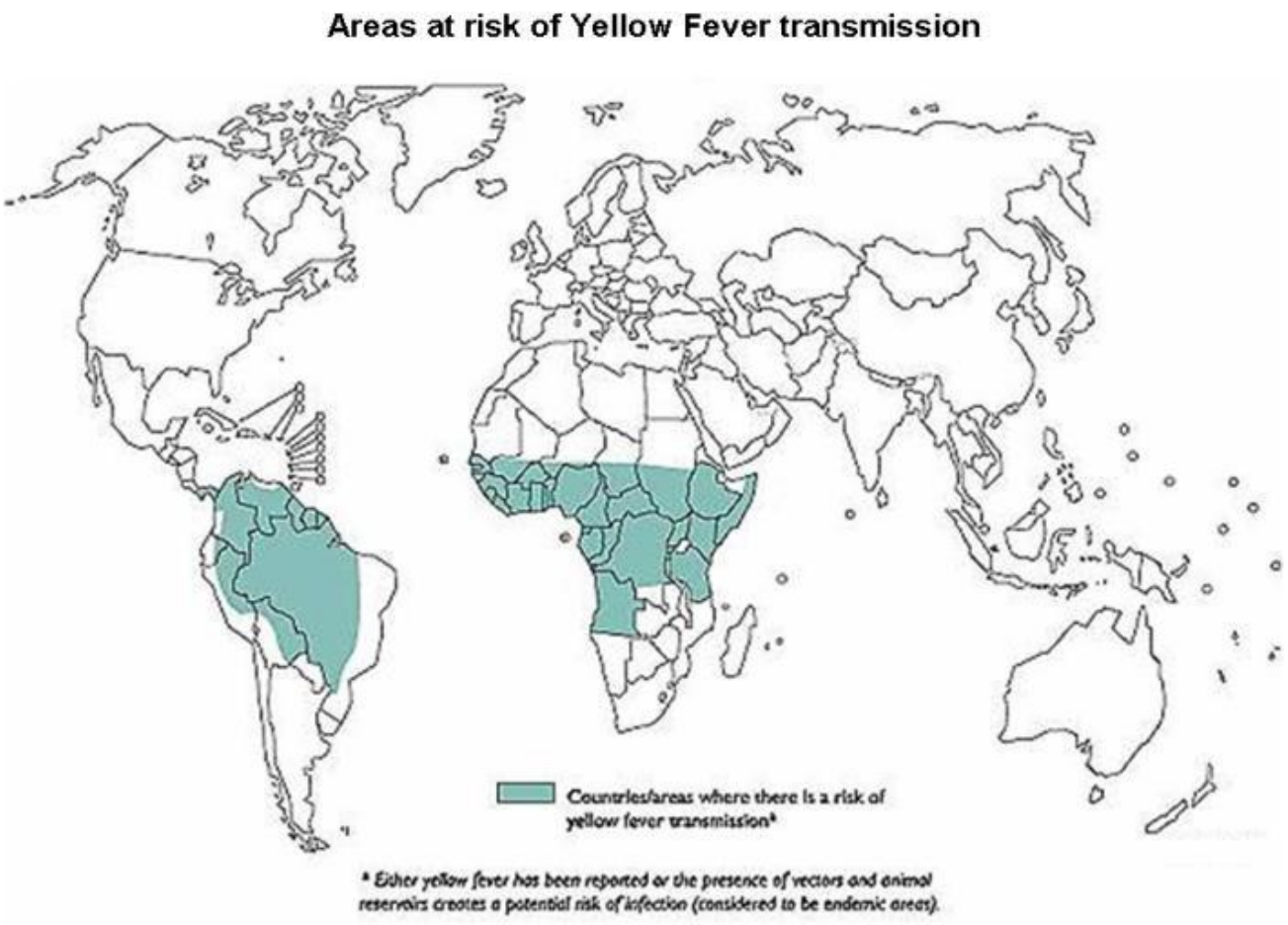


Figure 6: Yellow fever risky areas of South America and Africa

(WHO, 2018b)

2.5.3. Yellow fever in Ethiopia

The YF epidemic history in Ethiopia goes to the 1960s where still it is at risk among the African countries (Sérié *et al.*, 1968; Staples & Gershman, 2010). Since the first detection of the virus epidemic in Ethiopia, various research and outbreak investigations showed as the YFV is circulating in different locations of the country (Serie *et al.*,1968). Between 1960-1962, Ethiopia faced a large YFV outbreak mainly within the location of Gamo Gofa, Wollega, Jinka, and Kaffa resulted in 30,000 deaths, and 100,000 infections of a case fatality rate estimated to 30%. During the outbreak, mores cases of YF were seen in males and adults than females and children. Likewise, another outbreak occurred in Arba-Minch, east of

Lake Abaya, where it has not occurred in the previous outbreak in 1966 and resulted in the death of 450 and 2200 infections (Serie *et al*., 1968). In 2013 YF outbreak re-occurred in the South Omo Zone, the Southern part of Ethiopia's and resulted in many deaths (Lilay *et al*., 2017). Recently, another YF outbreak was reported from Gurage and Wolayita areas of South parts of Ethiopia (WHO, 2020c).

2.5.4. Life cycle and hosts of yellow fever

In its character, an acute viral infectious disease is transmitted to humans through the bite of infected mosquitoes. This virus involves non-human primates (animals). The hosts of YF include both humans and monkeys. The cycle of YF transmission occurs as follows: a tree-hole breeding mosquito bites an infected monkey, then it acquires the virus and can pass the virus on to any number of other monkeys that it may bite (Yellow Fever, 2020). Since there are natural animal hosts, the eradication of yellow fever is impossible currently (WHO, 2017b).

According to the CDC report, the life cycle of YF is accomplished in three ways, (1) jungle (sylvatic), (2) intermediate (Savannah), and (3) urban. The jungle (sylvatic) cycle involves the transmission of the virus between non-human primates (e.g., monkeys) and the potentially various mosquito species found in the forest canopy. The jungle cycle occurs in the tropical rainforests of Africa and South America. Mosquitoes from monkeys to humans transmit the virus when humans are visiting or working in the jungle. In most African situations, an intermediate (savannah) cycle exists involving the transmission of the virus from mosquitoes to humans living or working border areas of the jungle. In the savannah cycle, the virus can be transmitted from monkey to human or from human to human via mosquitoes in most Savannah areas of the African region, which is the most common in this region. The urban cycle involves the transmission of the virus between humans and urban mosquitoes that cause a serious outbreak. The virus is usually brought to the urban setting by a viremic human who was infected in the jungle or Savannah or through accidental transportation of infected mosquitoes (CDC, 2017; Gardner and Ryman, 2010) (figure 7).

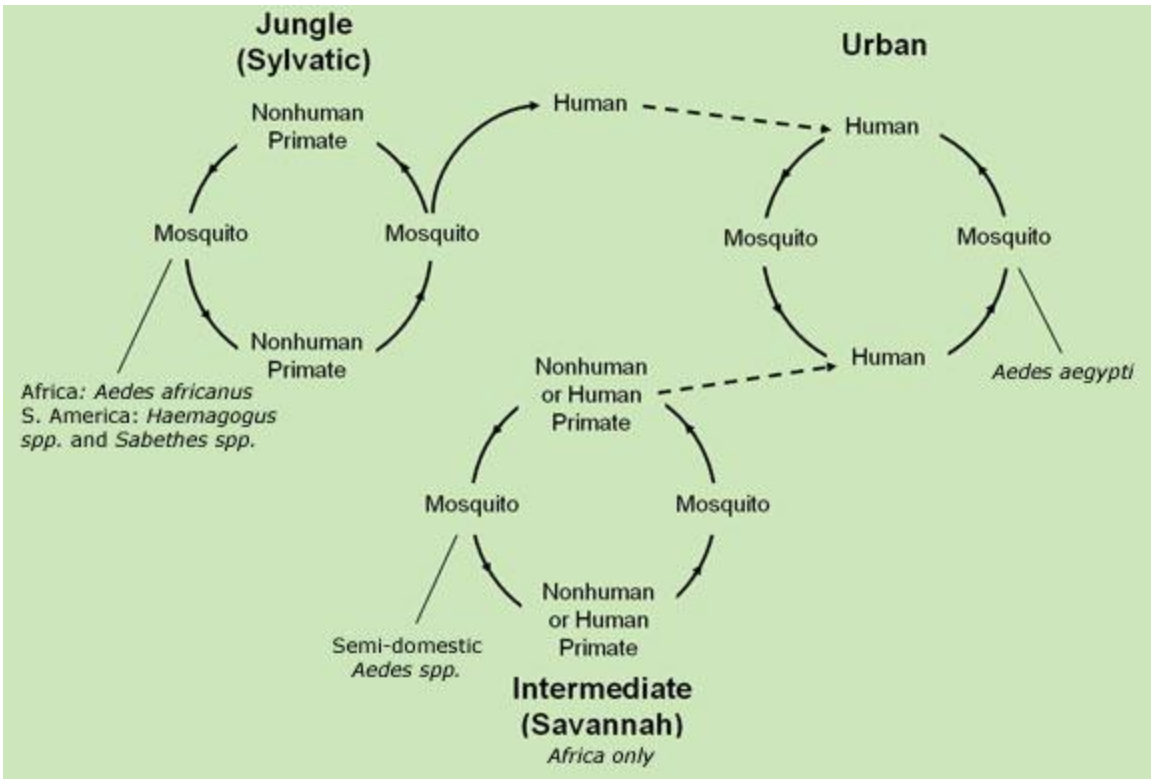


Figure 7. Illustration of the types of YFV life cycle

Source*:* (CDC, 2017)

2.5.5. Epidemiology of yellow fever in humans

Yellow fever virus can affect humans of all ages and gender across the globe as far as infected mosquito bites them, despite the level of exposure varies in between the groups (Johansson *et al*., 2010). In many studies, it was found a high prevalence of YF in males compared to females that deduced due to the increased movement and daily activities that lead them to exposure (Bundschuh *et al*., 2013). According to the WHO report, in an endemic area, the young are more affected compared to adults (WHO, 2019).

2.5.6. Epidemiology of yellow fever in animals

Yellow fever is mainly reserved and circulates in wild primates that potentially be sources for humans transmitted through the mosquitoes (de Jesus *et al.*, 2020). A seroepidemiological

survey for YF antibodies in domestic animals (camel, cattle, sheep, and goats) evidenced the detection of the viruses (Adu *et al.*, 1990).

2.6.7. Infection, pathogenesis, and clinical manifestations in humans of YFV

As for many other infectious diseases, YF infection presents with a broad spectrum of severity, with clinical presentation ranging from asymptomatic (in apparent) infection to fatal disease. The ratio of inapparent to apparent infection was estimated in field studies in Africa to approximate 7–12:1 (Monath, 2012). Direct studies on the pathogenesis of YF in humans are limited and much of the current knowledge is derived from comparative medicine studies in experimental models (Monath and Barrett, 2003). Wild-type YFV is primarily viscerotropic, with the liver being the most affected organ; however, the kidney, spleen, lymph nodes and heart, and probably other tissues are also injured by YFV. Many of the YF cases are mild and self-limiting, yet it can also be a life-threatening disease by causing hemorrhagic fever and hepatitis (where the term "yellow" came from jaundice it can cause).

In humans, the clinical sign ranges from its mildest form to a severe stage. In its mildest form, YF is characterized by sudden onset of fever and headache without other symptoms. Other patients experience an abrupt onset of a high fever (up to 104°F [40°C]), chills, severe headache, generalized myalgias, lumbosacral pain, anorexia, nausea, vomiting, and dizziness (Kemp, 1890; Knipe, 2007; Monath, 1987). The patient appears acutely ill, and examination might demonstrate bradycardia concerning the elevated body temperature (Faget's sign). The patient is usually viremic during this period, which lasts for approximately 3 days. Many patients have an uneventful recovery, but in approximately 15% of infected persons, the illness usually recurs in a more severe form within 48 hours following the viremic period. Symptoms include fever, nausea, vomiting, epigastric pain, jaundice, renal insufficiency, and cardiovascular instability where viremia generally is absent during this phase of symptom reactivation. A bleeding diathesis can occur, with hematemesis, melena, metrorrhagia, hematuria, petechiae, ecchymoses, epistaxis, and oozing blood from the gingiva and needle-puncture sites. Physical findings include scleral and dermal icterus, hemorrhages (e.g., hematemesis, melena, petechiae, ecchymoses), and epigastric tenderness without hepatic enlargement (Ayorinde *et al*., 2016; Gardner and Ryman, 2010).

2.5.7. Infection, pathogenesis and clinical manifestations of YFV in animals

Yellow fever virus has been shown to infect a variety of primate species. In African species, infection is associated with viremia but few clinical signs and is eliminated with rising neutralizing antibodies generally within a few days. YF wild hosts in the sylvatic cycle include the baboons, mangabeys, chimpanzees, red colobus monkeys (*Pilocolobus badius*), African green monkeys, and Patas monkeys. Viremia in these animals serves to propagate the virus in vector species and may initiate the urban cycle when transmission to peridomestic mosquitoes such as *A. aegypti* occurs. Once established in mosquito species that feed on humans, epidemics may become established in human populations (Wachtman & Keith, 2012).

2.5.8. Host immune response to Yellow Fever

The immune responses showing nearly the same in many studies with an effective production of the antibody for the virus vaccinated at different places of the world. IgM is the antibody produced after the infection or vaccination of a person by YFV (Gibney *et al*., 2010). Detailed examination showed that the humoral (neutralizing antibody) and cellular (CD8 and CD4 T cell) immune responses for YF are seen in the host immune system (Kongsgaard *et al*., 2017).

2.5.9. Diagnosis of Yellow Fever

This virus could be diagnosed according to the guideline of CDC generally can be accomplished by testing of serum to detect virus-specific IgM and neutralizing antibodies. Sometimes the virus can be found in blood samples taken early in the illness. In fatal cases, nucleic acid amplification, histopathology with immunohistochemistry, and virus culture of biopsy or autopsy tissues can also be positive (CDC, 2015).

2.5.10. Control of Yellow fever

WHO stated the control of YF can be done through the elimination of mosquito breeding sites. The effective way to prevent the transmission of this disease, however, relies on vaccination of the population at risk (WHO, 2008). Other preventive measures that decrease the risk of YFV infection include clothes that cover the entire body, wearing insect repellant

and remaining in well-screened areas. Unfortunately, there is no specific treatment for YF. There are no specific medications to treat YFV infections; treatment is directed at symptomatic relief or life-saving interventions. Rest, fluids, and the use of analgesics and antipyretics may relieve symptoms of fever and ache (Gershman *et al*., 2012). If there is an associated bacterial infection then it should be treated with the appropriate antibiotic (WHO, 2008).

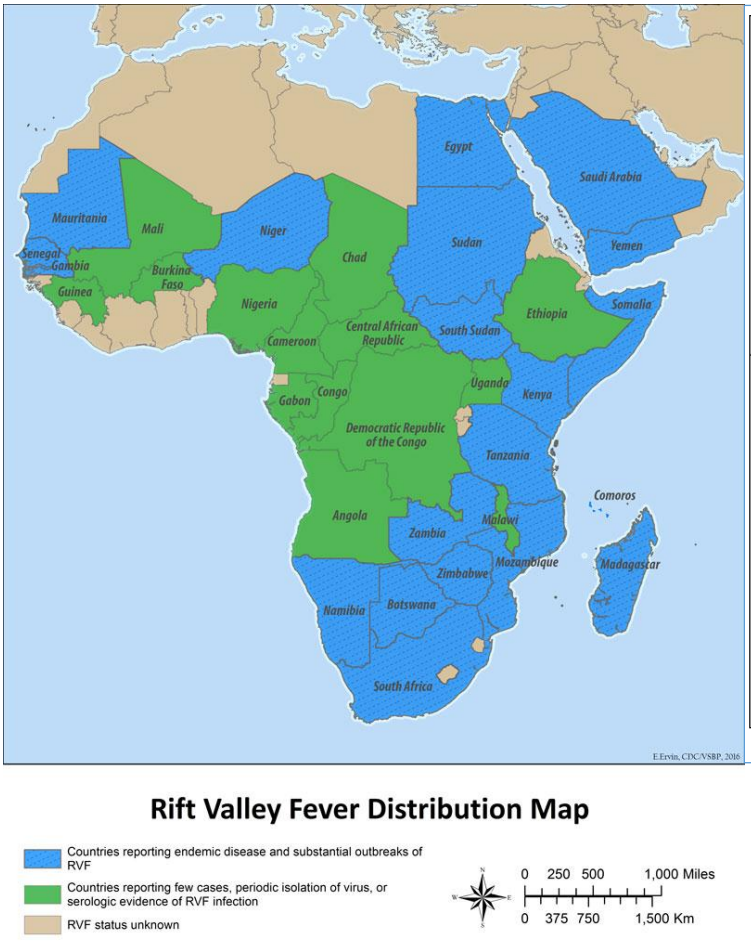
2.6. Rift Valley Fever

2.6.1. Biology of Rift valley fever

Rift valley fever virus is a family *Bunyaviridae* and a member of the *Phlebovirus* genus. In structure, the virions are enveloped and contain 3 single-stranded RNA genome segments designated large (L), medium (M), and small (S) coding for the viral proteins (Swanepoel and Coetzer, 2004). The L and M segments are negative in polarity while the S segment is ambisense (positive and negative sense)(Giorgi *et al*., 1991). The functions of segments; the L-segment encodes the viral RNA polymerase used for replication and mRNA transcription, the M-segment encodes two glycoproteins (Gn and Gc) and a nonstructural protein that can be expressed by itself (Nsm1) or infusion with Gn (NSm2)(Gerrard & Nichol., 2007). In an assembled structure, the RVFV particles possess a lipid-bilayered, enveloped virion, with the surface of each particle (Ellis *et al*., 1977) and is composed of subunits of Gn and Gc heterodimers, forming an ordered icosahedral shell of 122 capsomers (Huiskonen *et al*., 2009).

2.6.2. Epidemiology of Rift Valley fever

Since the first examination in Kenya, various outbreaks have been seen in Sub-Saharan countries RVFV has been detected across Africa, from Senegal to Madagascar, and from Egypt to South Africa. In 2000, RVFV reached the Arabian Peninsula. Moderate or large outbreaks have been documented in the Horn of Africa (1989, 1997–1998, 2006–2007) were associated with widespread rainfall. For predicting RVF outbreaks in this area, a model based on several satellite-derived observations has been proposed(Anyamba *et al*., 2009; CDC, 2020b) (figure 8).



Countries reporting endemic disease and substantial outbreaks of RVF	Egypt, the Gambia, Kenya, Madagascar, Mauritania, Mozambique, Namibia, Saudi Arabia, Senegal, South Africa, South Sudan, Sudan, Tanzania, Yemen, Zambia, Zimbabwe, Comoros
Countries reporting few cases, periodic isolation of virus, or serologic evidence of RVF infection	Angola, Botswana, Burkina Faso, Cameroon, Central African Republic, Chad, Democratic Republic of Congo, Ethiopia, Gabon, Guinea, Mali, Niger, Nigeria, Republic of Congo, Somalia, Uganda

Rift Valley Fever Distribution Map

Countries reporting endemic disease and substantial outbreaks of RVF

Countries reporting few cases, periodic isolation of virus, or serologic evidence of RVF infection

RVF status unknown

0 250 500 1,000 Miles

0 375 750 1,500 Km

Figure 8. Map of Rift valley fever distribution source

(Based on 2016 data): (CDC, 2020b)

In 1977, an explosive outbreak was reported in Egypt. The RVF virus was then introduced to Egypt via infected livestock trade along with the Nile irrigation system. During 1997–98, another major outbreak occurred in Kenya, Somalia, and Tanzania just following an El Niño event and extensive flooding across the region (Himeidan *et al*., 2014). Due to the trade of infected livestock commonly from the horn of Africa, RVF spread in September 2000 to Saudi Arabia and Yemen, marking the first reported occurrence of the disease outside the African continent that raised a concern of extending to other parts of Asia and Europe. The RVFV could result in human infection mainly from direct or indirect with the blood or organs of infected animals (Gershman *et al*., 2012). A human can acquire the virus through the handling of animal tissue during slaughtering or butchering, assisting with animal births,

conducting various veterinary procedures, or from the disposal of carcasses or fetuses(Archer *et al*., 2013). Hence, certain occupational groups such as herders, farmers, slaughterhouse workers, and veterinarians are supposed to be at higher risk of infection (Paweska, 2008)

2.6.3. Life Cycle and hosts of rift valley fever virus

The life cycle of RVF has two known cycles called the sylvatic cycle and domestic. The sylvatic cycle is maintained directly by multiple mosquito vectors and perhaps some combination of mammal hosts in varied topography. The full complement of sylvatic unknown but could include ruminants and rodents as important hosts and perhaps bats to a lesser degree too. *Aedes/Culex* mosquitoes act also as bridge vectors for the virus to livestock or less commonly directly to humans. Animal husbandry most frequently exposes humans to RVFV infection. There is also a direct transmission from mosquitoes to humans as a distinctly viable route of infection (Ahmed, 2010; Balenghien *et al*., 2013; Zeller *et al*., 1997; Infection Landscapes, 2012) (figure 9).

The RVFV is a zoonotic disease that affects ruminants and is characterized by high rates of abortion and death in young and adult animals. The virus also affects human beings where the symptoms are usually mild. Whenever the cases are severe hemorrhages, meningoencephalitis, retinopathy, and death can occur (Swanepoel and Coetzer, 2004). Studies showed that animals are first affected than human beings (Nicoletti, 2014).

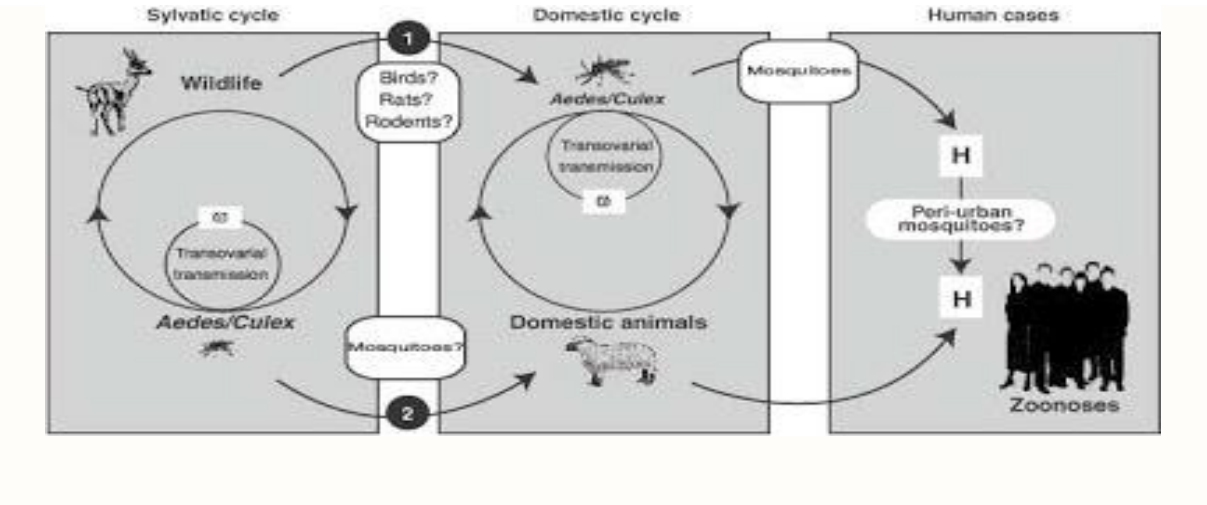


Figure 9. The life cycle of Rift Valley Fever

source (Infection Landscapes, 2012).

Aedes mosquitoes are primarily responsible for maintaining endemicity in sylvatic and domestic animal populations due to transovarial transmission and relatively low viremia among mammalian hosts leading mosquitoes to act as the primary natural reservoir for RVFV. This cycle is unusual for arthropod-borne viruses as there is typically a vertebrate host in the environment that serves as a natural reservoir (Ahmed, 2010; Balenghien *et al*., 2013; Zeller *et al*., 1997; Infection Landscapes, 2012).

Primarily, the RVFV is transmitted between ruminants through the bites of mosquitoes of numerous genera and species. In the case of humans, in addition to infection through these vectors, they are also infected by the contact or inhalation of aerosols generated when handling sick or dead infected animals or their fresh tissues(Davies *et al*., 1985). The lifecycle is described in figure 10 (Mansfielda *et al*., 2015).

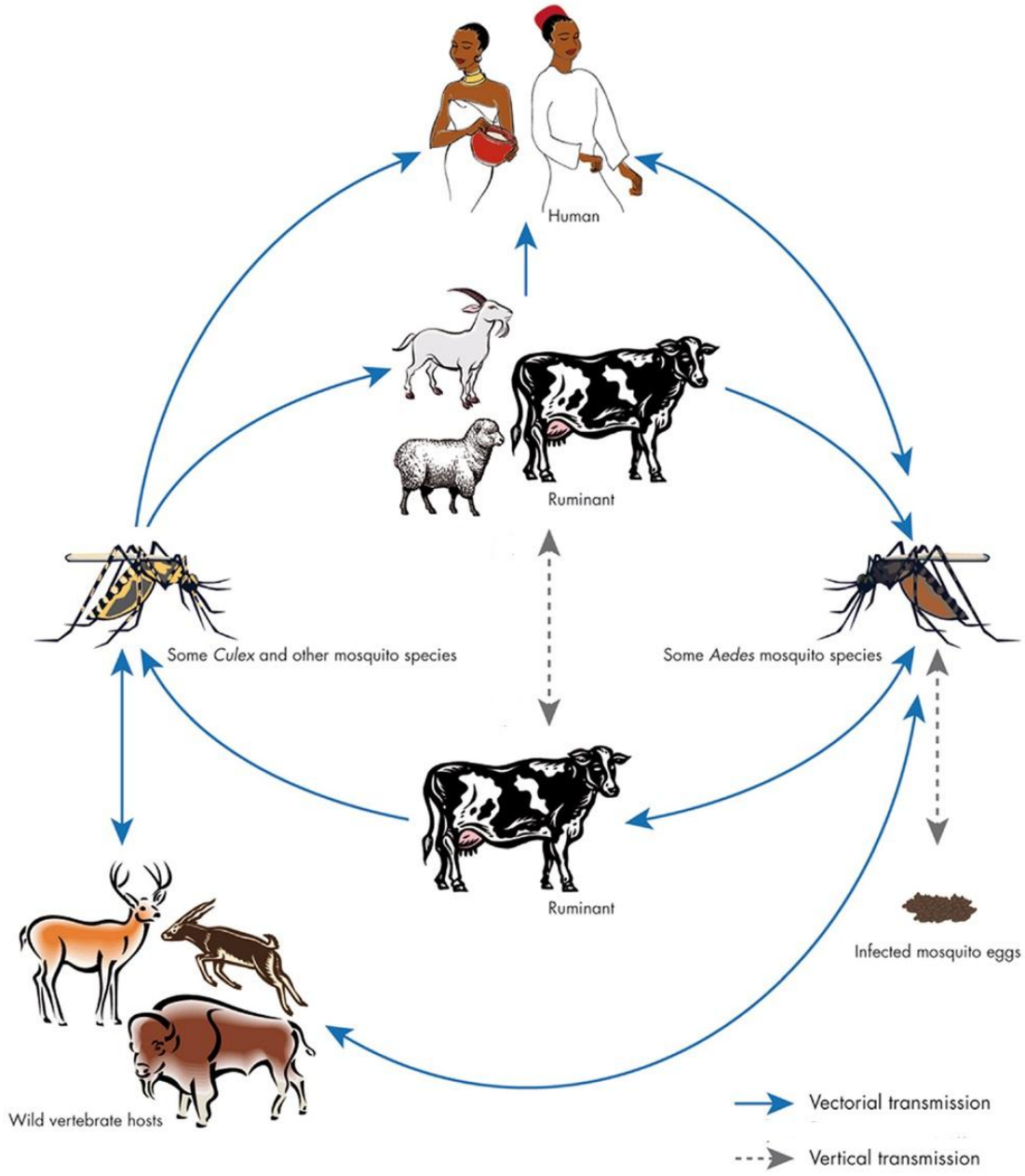


Figure 10. Hosts of Rift valley fever virus

(Mansfielda *et al*., 2015)

2.6.4. Infection, pathogenesis and clinical manifestations in humans and animals

The RVF infection, replication, and transmission involve complex interactions between the virus and various cells/tissues/organs of the vector. The successful transmission of the RVFV requires the ingestion of the virus to the vector midgut. This happens after the vector ingested a viremic blood meal from a host. Then the virus must enter the epithelial cells of the midgut, replicate and escape from the midgut cells into the hemolymph. This is followed by infection of secondary organs, including the salivary glands, where the virus enters the saliva and can then be transmitted to a new host (Hardy, 1998).

In many cases, patients with RVF show or suffer from a self-limiting, febrile illness. Nonetheless, some other patients develop pathological forms such as neurological disorders, vision loss, hemorrhagic fever, or thrombosis as shown in the following chart (Ikegami and Makino, 2011) (figure 11).

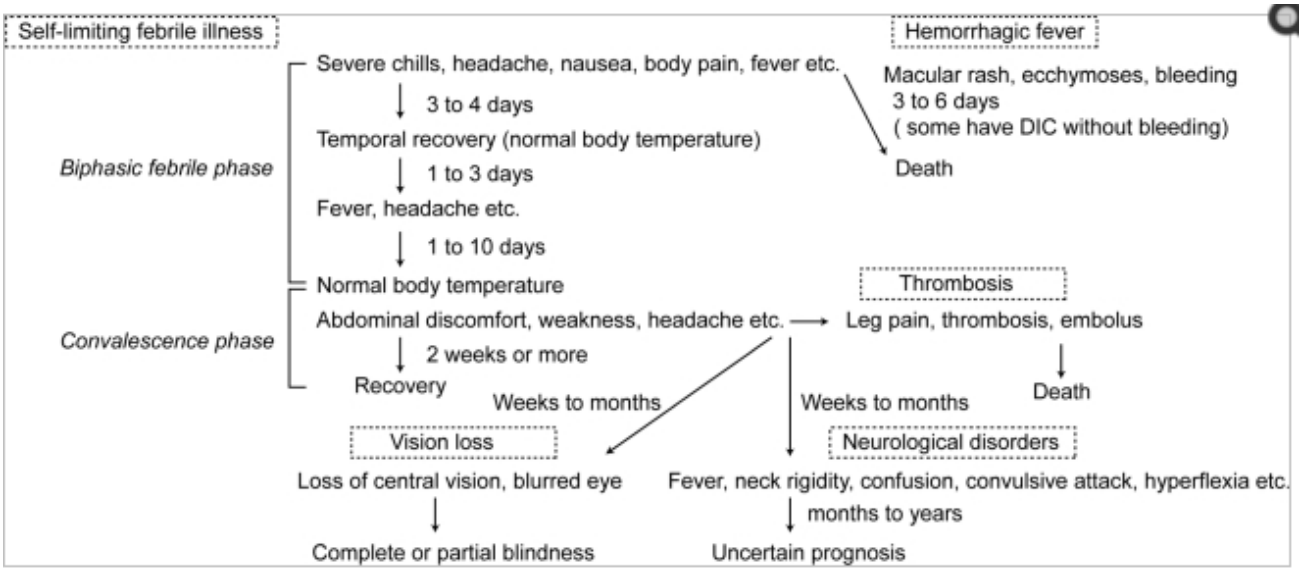


Figure 11. Pathogenesis of rift valley fever

(source: (Ikegami and Makino, 2011).

Typically, people infected with RVF show clinical sign such as self-limiting febrile illness, neurological disorders, vision loss, hemorrhagic fever, thrombosis, and the possibility of

vertical transmissions (Abdel-Aziz *et al*., 1980; Alrajhi *et al*., 2004; Daubney *et al*., 1931; Deutman and Klomp, 1981; Kitchen, 1934; Maar *et al.*,1979; Mundel and Gear, 1951; Smithburn, and Mahaffy, 1949).

2.8.4. Infection, pathogenesis and clinical manifestations animals

The clinical manifestations depend on the age and pregnancy of affected animal species During epidemics, the occurrence of numerous morbidity and mortalities among young animals is characteristic. Signs such as severe illness, abortions are characteristics in animals. Pregnant sheep and cattle affected by this disease will usually abort (80-100%)(OIE, 2018).

The pathological changes after RVFV infection of mice mimic the pathology findings in newborns' lambs, hence they show, ruffed hair, death after encephalitis, paralysis, liver lesions (coagulative necrosis), and depletion of glycogen in hepatocytes (Smith *et al*., 2010).

2.6.5. Host immune response for Rift Valley Fever

The innate and adaptive immune responses contribute to the clearance of RVFV in infected hosts (Bird *et al*., 2009; do Valle *et al.*, 2010). The evidence so far for the role of innate immunity is frequently based on results from experimental models (Bouloy *et al*., 2001; do Valle *et al*., 2010; Morrill *et al*., 1990; Morrill *et al*., 1991). Interferon-alpha (IFN-α) is believed to protect against RVFV because monkeys that secreted this cytokine within 12 h of being challenged with RVFV did not develop the disease (Morrill., *et al.*, 1990). However, RVFV non-structural (NSs) protein inhibits IFN-α and IFN-β production/induction, thereby enabling early replication and viremia (Bouloy *et al*., 2001; Ikegami *et al*., 2009). Anti-RVFV antibodies are detectable 4 to 8 days following infection(Paweska *et al.*, 2005a; Paweska, *et al.*, 2005b; Williams *et al.*, 2011). Neutralizing antibodies are believed to be crucial for the protection of infected hosts (Morrill *et al.*, 1990; Pepin *et al*., 2010b). The innate immune systems activate the IFN and signal T cells and B cells to begin the translation to an adaptive immune response (Paul, 2008).

2.6.6. Diagnosis of rift valley fever

2.6.6.1. Clinical diagnosis of rift valley fever

Early detection of suspected cases is crucial to ensure timely control measures to be applied to lessen the disease burden. Commonly a sudden onset of large numbers of abortions (‘abortion storms’) and mortalities among young animals in affected livestock, together with the appearance of the disease in humans, is considered characteristic of an RVF epidemic (Infection Landscapes, 2012).

2.6.6.2. Serologic diagnosis of rift valley fever

In the early stage of illness in the blood and postmortem tissue, the virus may be detected by antigen-detection ELISA. Antibody testing using an enzyme-linked immunoassay (ELISA) can be used to confirm the presence of IgM antibodies, which appear as an early, transient response, and IgG antibodies, which persist for several years. Both IgM and IgG antibodies are specific to the RVFV (CDC, 2020a).

2.6.7. Control and prevention of rift valley fever

Due to the economic impacts of the disease are devastating, timely surveillance to monitor for RVF infection in animal populations and immediate notification upon detection are essential elements for the prevention and control of RVF. Controlling the vector (mosquito) population through spraying and management of breeding grounds has also been an effective mechanism. Systems used to monitor variations in climatic conditions can provide warning of impending conditions that favor the flourishing of mosquito populations and signal the need to implement enhanced control measures (OIE, 2018).

Vaccination can be used for the prevention of RVF in animals in areas where the disease is endemic. A modified live vaccine is available that requires only one dose and produces long-lived immunity, but is not recommended for pregnant animals due to the risk of abortion. The inactivated RVF vaccines, also widely and successfully used do not cause unwanted effects,

but these are more expensive to produce and require multiple doses to produce protective immunity (Nielsen *et al*., 2020).

Nowadays an inactivated vaccine has been developed for human use. However, this vaccine is not licensed or available commercially but has been used experimentally to protect veterinary and laboratory personnel at high risk of exposure to RVF. Natural immunity will develop in humans who have contracted RVF and recovered from the disease (Rusnak *et al.*,2011). Personal protective clothing, such as long shirts and trousers, use bed nets and insect repellent, and avoid outdoor activity at peak biting times of the vector species is an effective measure. Care must be taken when handling sick animals or human patients, their tissues, and samples (Paweska and Vuren, 2013).

2.7. Chikungunya virus

2.7.1. Biology of chikungunya virus

The CHIKV is a re-emerging mosquito-borne viral disease transmitted by *Aedes* mosquitoes that mostly results in acute and chronic articular cases in humans *(*Lumsden, 1955*;* Simon *et al*., 2008). CHIKV is a positive single-stranded (ss) RNA virus that belongs to A*lphavirus* (IV) genus of the family *Togaviridae*. It belongs to the Semliki Forest Virus (SFV) complex. The virus "chikungunya" is derived from a word in the Swahili or Makonde/Kimakonde language, meaning "to become contorted/that which bends up" showing the suffers within joint pain. *Alphaviruses* can be broadly divided into New World and Old World viruses (Peters and Dalrymple, 1990; Rulli *et al*., 2005). Both viruses group have their distinct ways in which they interact with their respective hosts and variability in their pathogenicity, cellular and tissue tropism, cytotoxicity, and interference with virus-induced immune responses. The virus genome encodes two open reading frames the 5′ end encodes the virus non-structural proteins (NSP) 1–4, which together form the virus replicase, whereas the 3′ ends encode the capsid and the envelope glycoproteins (Solignat *et al*., 2009).

2.7.2. Epidemiology of chikungunya virus

CHIKV was first identified in southern Tanzania in 1952 and then occasionally causes outbreaks in Africa and Asia (Robinson, 1955; Simon *et al*., 2008). Following this, it is being reported in many parts of the world. Between the 1960s and 1990s, the virus was repeatedly reported from numerous countries in Central and Southern Africa, including Sudan, Uganda, the Democratic Republic of Congo, the Central African Republic, Malawi, Zimbabwe, Kenya, and South Africa (Powers and Logue, 2007). CHIKV has been also isolated in Western African countries, including Senegal, Benin, the Republic of Guinea, Côte d'Ivoire, and Nigeria (Lumsden, 1955; Schwartz and Albert, 2010).

In Southeast Asia, frequent outbreaks were reported from the 1960s through to 2003 in India, Malaysia, Indonesia, Cambodia, Vietnam, Myanmar, Pakistan, and Thailand (Powers and Logue, 2007). Indeed, numerous cities, including Bangkok and Calcutta identified as particularly active sites of CHIKV transmission and disease cases (Powers and Logue, 2007).

Since the beginning of 1986, CHIKV outbreaks resurged with major clusters documented in Senegal (1986, 1996, and 1997), Côte d'Ivoire (1996 and1997), Democratic Republic of Congo (1998-2000), Indonesia (2003), Kenya (2004), Comoros (2005), the Seychelles, Mauritius, Madagascar and Réunion islands (2005-2006), and India (2006 and 2007) Powers and Logue, 2007).

CHIKV cases have been also reported in Europe (United Kingdom, Belgium, Germany, Czech Republic, Norway, Italy, Spain, and France), Hong Kong, Canada, Taiwan, Sri Lanka, and the United States; however, these were directly associated with the return of tourists from India and the affected islands of the Indian Ocean (Pardigon, 2009; Powers and Logue, 2007). Currently, chikungunya fever is reported in over 60 countries from Asia, America, Europe, and Asia (WHO, 2020a).

There are 2 epidemiological transmission cycles of CHIK fever: a sylvatic cycle, occurring primarily in Africa mainly between wild primates and arboreal *Aedes* mosquitoes (Chhabra *et al*., 2008; Powers and Logue, 2007), where humans are accidental hosts; and an urban

human-mosquito-human transmission cycle that typically occurs in cities in Asia (Chhabra *et al*., 2008).

Including Africa, the virus identified in Asia, and the Indian subcontinent, Europe, and the American region(Pan American Health Organization, 2015). The CHIKV Phylogenetic sequence analyses indicate that the CHIKV originated in Africa over 500 years ago (Volk *et al*., 2010) (figure 12).

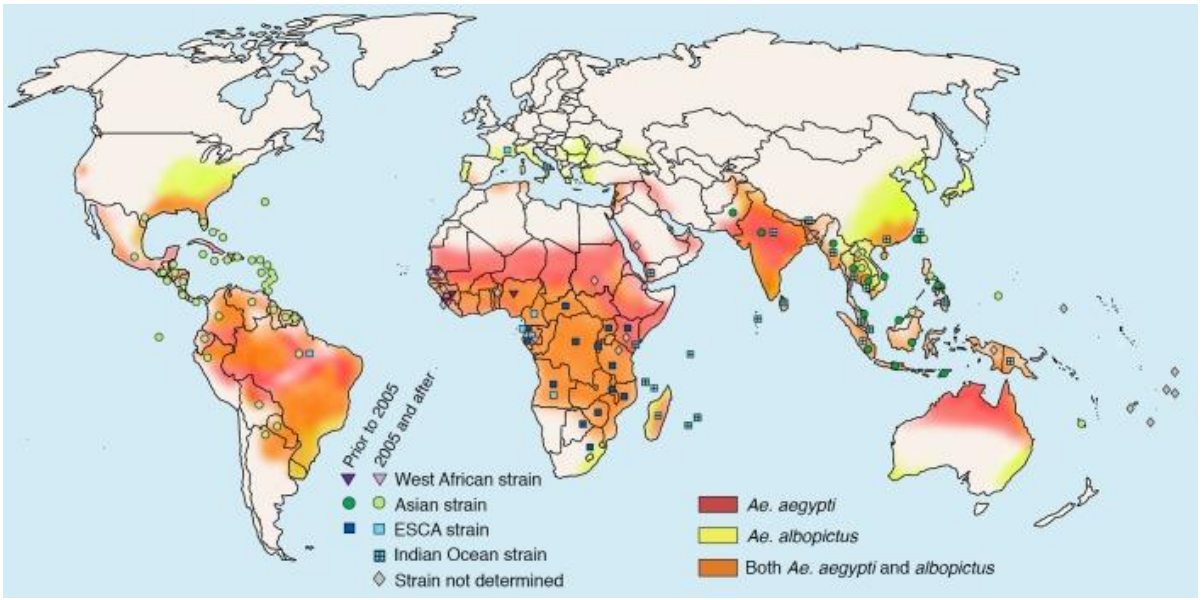


Figure 12. Map showing the geographic distribution of endemic CHIKV and its primary vectors, *Ae*. *aegypti* and *Ae*. *albopictus*

Source*: (*Powers *et al.,* 2000*;* Volk *et al*., 2010*).*

2.7.3. Life cycle and hosts of chikungunya virus

CHIKV is maintained in a sylvatic cycle among forest-dwelling *Aedes* spp in many Africa settings. mosquitoes, wild primates, squirrels, birds, and rodents (Diallo *et al.*, 1999). The sylvatic cycle uniquely maintains the CHIKV in Africa that comprises non-human primates and different forest-dwelling mosquitoes species including *Aedene* mosquitoes (*Ae. Africanus, Ae. furcifer-taylori, Ae. dalzieli,* etc.,) and non *Aedene* mosquitoes (*Mansonia*,

Culex, etc.)(Diallo *et al*., 1999). The current understanding of sylvatic transmission is not clear, where involvement of other species as reservoirs might occur (Sam *et al.*, 2015).

The main vectors of CHIKV in Asia are *Ae. aegypti* and *Ae. Albopictus* (Kumar *et al*., 2008). Transmission in Asia occurs in an urban cycle whereby the mosquito spreads the disease from an infected human to an uninfected human (Jain *et al*., 2008). Hence, CHIKV is transmitted to humans from infected non-human primates and other humans by the bite of *Aedes* mosquitoes (Weaver, 2006). Evidence exists that CHIKV can also be passed from an infected mother to a developing fetus (Chhabra *et al*., 2008; Pardigon, 2009). Furthermore, inhalation of aerosolized CHIKV in a laboratory setting may lead to CHIKV infection (Scherer *et al*.,1980). Transmission in the epidemic area is primarily through a human–mosquito–human cycle involving the vectors; *Aedes aegypti* and *Aedes albopictus* mosquitoes (Powers *et al.*, 2000). Fundamentally, the virus is assumed to be maintained in nature by circulation in arboreal primates (Sam *et al*., 2015) (figure 13). The host of CHIKV ranges mainly from humans (Powers *et al*., 2000; Robinson, 1955) to non-human primates, rodents, and birds (Pardigon, 2009; Pialoux *et al*., 2007).

The common transmission cycle is human-to-mosquito-human transmission well established. In endemic areas of tropical and subtropical regions of sub-Saharan African and South East Asia, two common transmission cycles are documented; the rural and the urban transmission cycles. In the rural enzootic cycle, the transmission occurs between the forest or savanna *Aedese* (*Stegomyia*) and animal reservoirs (Diallo *et al*.,1999; Jupp and McIntosh, 1990; Jupp *et al*., 1981) where the nonhuman primates being the presumed major reservoir hosts (Inoue *et al*., 2003; Thiberville *et al*., 2013). A study conducted from Bangkok and the surrounding alluvial plain of the Chao, Phyra River showed a haemagglutination-inhibiting (HI) and neutralizing antibodies to chikungunya in adult cattle, water buffalo, horses, pigs, dogs, monkeys, rabbits, and bats yet, no antibodies were found in cats urban rodent species (Halstead and Udomsakdi, 1964).

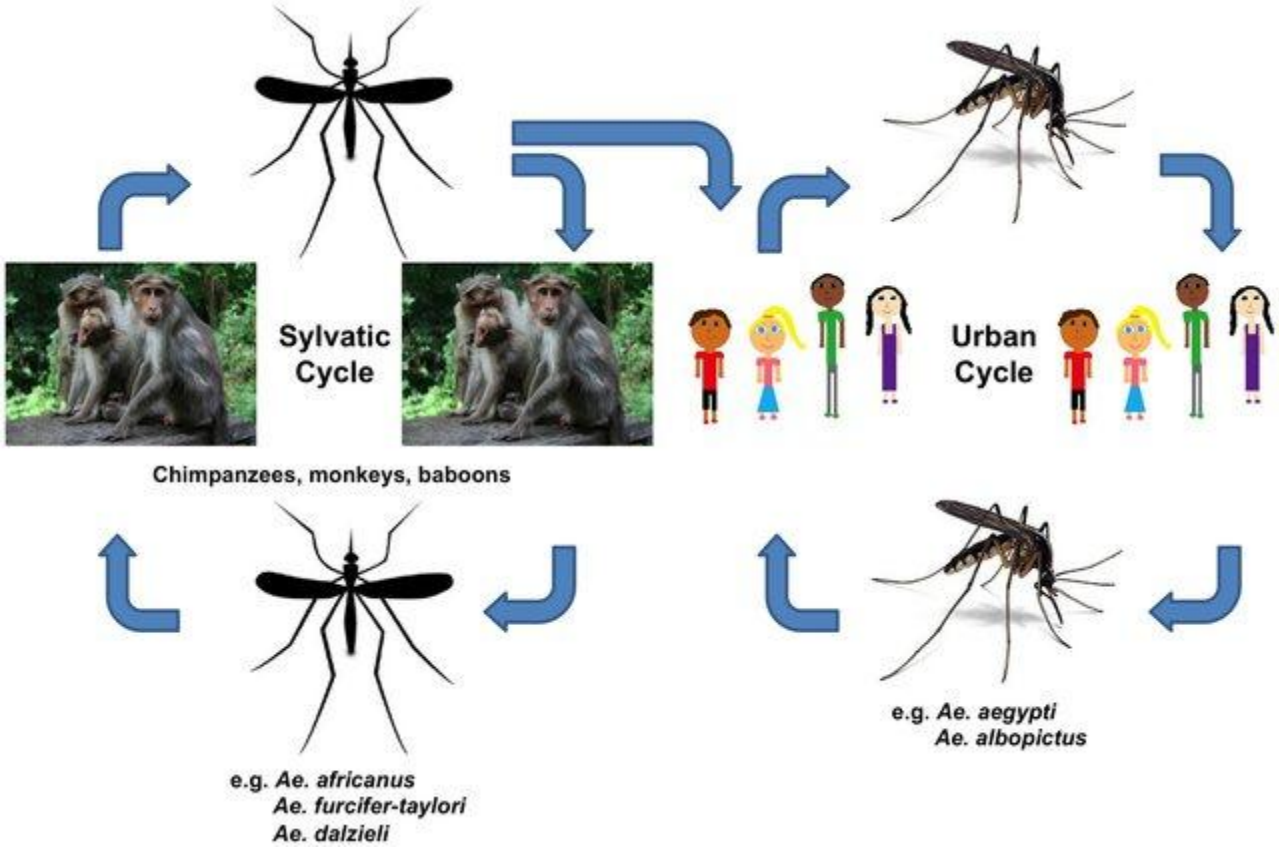


Figure 13. The life cycle of Chikungunya virus.

(Diallo *et al.,* 1999).

2.7.4. Infection, pathogenesis and clinical manifestations in humans

The CHIKV RNA genome encodes the two open reading frames. T Transmitted via mosquitoes of the *Aedes* genus, CHIKV replicates in the dermal site of inoculation and is then disseminated to other parts of the body. The CHIKV with an incubation period of 2–4 days is followed by a sudden onset of clinical symptoms that include high fever (usually > 38.5°C), rigors, cephalgia, myalgia, petechial or maculopapular rashes, and often incapacitating arthralgia (Mourya & Mishra, 2006; Yazdani & Kaushik, 2007). The acute phase viremia could have a viral load of 109 –1012 viral particle ml-1 , and a strong type 1 interferon (IFN) response with the production of inflammatory cytokines is usually observed (Ng *et al*., 2009; Schilte *et al*., 2010). Acute symptoms are typically resolved within 2 weeks.

Some of the *Alphaviruses* are not pathogenic to humans, whereas others are highly infectious, with the associated clinical diseases ranging from mild to severe. Most alphaviral infections in humans and domesticated animals are "dead-end" where the virus cannot be transmitted to a new host; hence, the evolutionary pressure driving viral diversification may be linked to their true host species (Griffin, 2007*;* Powers *et al.*, 2001; Weaver and Reisen, 2010b).

From a clinical perspective, *Alphaviruses* are subdivided into those associated with encephalitis (predominantly New World viruses) and those associated with polyarthritis and a rash (predominantly Old World viruses). CHIKV particularly infects stromal cells of the central nervous system, the lining of the choroid plexus. Hence, it is common to see cases of meningoencephalitis (primarily in neonates) and hemorrhagic disease than the arthritogenic cases (Griffin, 2007*;* Powers *et al.*, 2001; Weaver and Reisen, 2010b).

During epizootic cycles, the CHIKV cycle mainly circulates with human-mosquito-human that may maintain the virus during the interepidemic period (Chevillon *et al.*, 2008*;* Mohan, 2006*;* Pialoux *et al*., 2007; Powers and Logue, 2007; Simon *et al*., 2008). In epidemics situations, human beings serve as the CHIKV reservoirs; whereas; during interepidemic periods, several vertebrates, such as monkeys, rodents, birds, are identified as the reservoir hosts. Wild CHIKV maintenance in Asia is unknown. There is no evidence for transovarial transmission of CHIKV in mosquitoes (Sam and AbuBakar, 2006). Documents show a vertical maternal-fetal transmission of CHIKV in pregnant women.

Common symptoms of CHIKV infection include high fever, rigors, headache, photophobia, and a petechial rash or maculopapular rash. Besides, most infected individuals complain of severe joint pain that is often incapacitating (Mourya and Mishra, 2006; Yazdani and Kaushik, 2007). A person with Chikungunya fever (CHIKF) may reach a high-grade fever (102-105°F/ 39 to 40 °C) (Chevillon *et al*., 2008; Mohan, 2006; Mohan and Sharma, 2007; Pialoux *et al*., 2007; Powers and Logue, 2007; Simon *et al*., 2008; Sudeep and Parashar, 2007). Other signs that might be observed in affected person include; abdominal pain, constipation, cervical, or sometimes generalized lymphadenopathy (Bandyopadhyay and Ghosh, 2008; Inamadar *et al*., 2008), arthropathy (Powers and Logue, 2007), direct impact on

pregnancy with a higher risk of abortion in the first trimester and mother-to-child transmission in the last trimester *(*Powers and Logue, *2007; Simon et al*., 2008), hemorrhagic manifestations of fulminant hepatitis (Economopoulou *et al*., 2009) and less frequent manifestations of myocarditis after febrile illness may be observed. Other less include acute febrile illness (Obeyesekere and Hermon, 1972).

In general, CHIKF affects all age groups, and both genders are equally affected. The incubation period variably ranges from 3 to 12 days (usually 3-7 days). In susceptible populations, the attack rates can be as high as 40-85% (Chevillon *et al*., 2008; Mohan, 2006; Mohan and Sharma, 2007; Pialoux *et al*., 2007; Powers and Logue, 2007; Simon *et al*., 2008; Sudeep and Parashar, 2007).

2.7.5. Infection, pathogenesis and clinical manifestations in animals

In nonhuman primates, the CHIKV infection recapitulated the viral, clinical, and pathological features observed in human disease. In the macaques, long-term CHIKV infection was observed in joints, muscles, lymphoid organs, and liver, which could explain the long-lasting CHIKV disease symptoms observed in humans (Labadie *et al*., 2010).

2.7.6. Host immune response for chikungunya fever

The innate immune response against the virus consists of macrophages, dendritic cells (DCs), and natural killer cells (NKs) and is followed by the activation of B and T lymphocyte-mediated adaptive immune response. The subsequent generation of memory cells leads to a specific response to the viral infection and protects from reinfection. Pathogen-specific, humoral, and cell-mediated immune responses that together constitute adaptive immunity are carried out by B and T lymphocytes, respectively. In a mouse model, induction of anti-CHIKV antibodies that subsequently led to rapid clearance of the virus was demonstrated. Following that, B cell (μMT) knockout mice showed a more severe disease and persistent viremia (for over a year) highlighting the importance of these antibody-producing cells for CHIKV clearance (Lum *et al*., 2013). In mice that lack both B and T cells, prophylactic

administration of anti-CHIKV monoclonal antibodies was sufficient to prevent virus persistence (Hawman *et al*., 2013). Besides, therapeutic administration of a human neutralizing monoclonal antibody in rhesus monkeys on days 1 and 3 after CHIKV infection blocked virus spread and inflammation in several tissues including joints and muscles (Broeckel *et al*., 2017). In humans, anti-CHIKV IgG is first detected in the early convalescent stage, when naturally acquired IgG response is dominated by the antibodies of IgG3 subtype. An early appearance of these antibodies correlates with protection against complications of chronic CHIKVD (Kam *et al*., 2012). The functional role of infection-induced specific IgM against CHIKV is less well-characterized compared to immune IgG during acute and early convalescent phases of infection in mice and humans (Amor *et al*., 1996).

2.7.7. Diagnosis of CHIKV

2.7.7.1. Serologic diagnosis of chikungunya virus

Alphavirus species can be characterized by a variety of serological methods (hemagglutination inhibition, ELISA, complement fixation, and neutralization of viral infectivity using reference serum samples) (Kucharz and Cebula-Byrska, 2014; Morrison, 2014). The recommended serum sample for CHIKV IgG antibody is fourfold increase taken during the acute and recovery phase is required to serodiagnosis, however, it is often problematic to collect paired samples. Including CHIKV and other arboviruses, utilize several serological assays have been developed, with high reliability and specificity. ELISA is used as a first-line serological technique used for CHIKV diagnosis and others include indirect immunofluorescence (IFA) assays, and the most suitable sample type is serum, generally using 1/100 dilutions (Prat *et al*., 2014).

In the serologic diagnosis of CHIKV, antigen detection would be a good alternative, as it makes use of more accessible, established serology platforms such as ELISA and immunochromatographic assays (ICAs). The diagnostic challenges for CHIKV are similar to that of dengue, another *Aedes*-borne viral infection. The diagnosis of dengue has been greatly aided by the recent introduction of NS1 antigen detection kits. On the other hand, there are

currently no widely existing CHIKV antigen commercial assays. Moreover, the performance characteristics of the CHIKV antigen detection ELISAs described to date are not clearly defined (Kashyap *et al*., 2010; Shukla *et al*., 2009). These reported assays also use whole virus antigen, which would require biosafety level 3 containments during preparation.

Serological diagnosis by detecting immunoglobulin M (IgM) or IgG seroconversion is more widely used, as it is relatively cheaper and easier to perform. A disadvantage of antibody testing is the possibility of cross-reactivity with other *alphaviruses*. Besides, as IgM may persist for months, a single raised IgM may indicate a recent past infection rather than an acute infection. Many reference laboratories have developed in-house assays for the detection of CHIKV IgM (Niedrig *et al*., 2009). In the case of antibody-based IgM ELISA is found to be cost-effective, but it takes 5 to 7 days for the patient to develop antibody after the onset of illness and, thus, has less implication for early clinical diagnosis and patient management (Lanciotti *et al*., 2007; Yap *et al*., 2010).

2.7.7.1.1. Enzyme-linked immunosorbent assay

ELISA immunocapture can detect CHIKV-specific IgM and IgG in serum. The IgM can be detected in patients against CHIKF, which commonly appears 3-8 days after the onset of infection that could persist in from months to years. The IgG might be detected in serum after 4-10 days from the onset of infection and may persist for years (Weaver and Lecuit, 2015) and possibly lifelong. The presence of IgM antibodies indicates a recent CHIKV infection(Burt *et al.*, 2012), generally occurring in samples taken 2 weeks before patients become symptomatic. Detection of specific IgG antibodies, on the other hand, indicates previous CHIKV infection, which can be recent or as long ago as several months or years, given the persistence of anti-CHIKV IgG antibodies (Weaver and Lecuit, 2015).

ELISA is a fast and sensitive system largely used for the detection of anti-CHIKV antibodies. The most common tests used for the diagnosis of CHIKV infection are IgM antibody-capture ELISA (MAC- ELISA) and indirect ELISA (i-ELISA) for the detection of type M (IgM) and type G (IgG) immunoglobulin, respectively (Cavrini *et al*., 2009). Despite the fact all the

advantages, ELISA is not, without its disadvantages: including; false-positives because of cross-reactivity with other alphaviruses (Ross River virus, Barmah Forest virus, and Sindbis virus (Pialoux *et al*., 2007) and its sensitivity being much reduced (4–20%) in serum samples taken during the acute phase (Blacksell *et al*., 2011).

2.7.7.1.2. Indirect immunofluorescence Assay (IFA)

This technique is accurate and reliable, which is widely used for the detection of specific anti-CHIK antibodies. IFA discloses the occurrence of type-specific antibodies against CHIKV by detecting the presence of virus antigens in infected cells. Commercially available IFA test specificity ranges from 75 to 100% in serum collected within 6 days after infection. The method has shortcomings, as it is laborious and needs special training. Besides, interpretation of the microscope examination results can be rather subjective, and absence of standardization between labs. Generally, ELISA or IFA results method is used for confirmation purposes (Yap *et al*., 2010).

2.9.5.1.3. Antibody neutralization assay (ANA)

The principle of ANA utilizes the interaction of viral antigens and specific antibodies to block infection. The neutralization test requires the mixing of virus and serum, and the resulting mixture is then inoculated into cell culture. Inhibition of the virus can then be tested, using a variety of methods, after several days (Sambri *et al*., 2013). The microneutralization assay (MNA) determines the neutralizing antibodies.

Even though ANA and MNA methods have high specificity and sensitivity, there are hindrances, namely, they are labor-intensive since only a few numbers samples can be processed in each run and a Biosafety Level 3 laboratory (BSL-3) is mandatory because the live virus is being used(Weber *et al*., 2014).

The shortcomings of antibody testing take account of cross-reactivity with other *Alphaviruses* and the problem of it not being able to distinguish between recent past and

acute infection, as well as the fact that its sensitivity varies between clinical settings (Henss *et al*., 2019).

2.7.8. Molecular diagnosis of chikungunya virus

Molecular diagnostic tests used for the detection of CHIKV include reverse transcription (RT) and amplification (PCR) assay of fragments. In RT-PCR amplification CHIKV replicates rapidly to high titers in the host, and viral RNA generally can be easily detected by real-time RT-PCR in the first week of acute infection after the onset of clinical illness (Lanciotti *et al*., 2007). Nested and real-time PCRs molecular methods can also be used in the viral RNA detection of CHIKV from plasma or serum (or other) sample types (Gérardin *et al*., 2008). Molecular assays (TaqMan real-time PCR, RT-LAMP assay, and reverse transcription PCR) are more sensitive (Reddy *et al*., 2012) in the early stage of CHIKF (2–5 days) when CHIKV-specific IgM is not yet detectable. In the later stages of CHIKF (>5 days), CHIKV-specific IgM is a more reliable indicator. Other methods included under this category were Loop-mediated isothermal amplification (LAMP) (Reddy *et al*., 2012), microfluidic lab-on-chip integrating multiplex molecular amplification, and DNA microarray hybridization (Tan *et al*., 2014).

2.7.9. Clinical diagnosis of chikungunya virus

There are three phases of clinical diagnosis of symptomatic CHIKV infection: acute, post-acute, and chronic. The acute phase is considered to occur in the first 3 weeks of clinical manifestations with signs of high fever, and malaise that usually lasts 7-10 days (Burt *et al*., 2012; Weaver and Lecuit, 2015). After 2-5 days from the onset of fever, bilateral and symmetric polyarthralgia that involves multiple joints, hands, wrists, ankles, and axial skeleton start to occur. The pain after this acute case may be intense and leading to immobilization. Patients would also show rash and pruritus (Taubitz *et al*., 2007). In most cases, full recovery of patients is common although few joint cases might indicate relapsing signs for several months (Schwartz and Albert, 2010).

In the post-acute phase, only a small proportion of patients remain completely asymptomatic 2–3 weeks after the onset of disease (Simon *et al*., 2015). Clinical manifestations observed

during the post-acute phase, which indicate the persistence of the initial inflammatory process, including arthritis/arthralgia, edematous polyarthritis of fingers and toes, morning pain and stiffness, and severe tenosynovitis (Parola *et al*., 2006).

A set of nonspecific clinical manifestations that are not always associated with CHIKV usually occurs, such as chronic fatigue, changes in skin color, alopecia, decompensated endocrine, and metabolic diseases, as well as the decompensation of other preexisting chronic diseases, depression, and anxiety (Simon *et al*., 2015).

Chronic phase: It is estimated that the percentage of patients infected with CHIKV who progress to the chronic phase (more than 3 months) varies from 40 to 80% (Chopra *et al*., 2012; Edington *et al*., 2018; Sissoko *et al*., 2009), and they may endure clinical manifestations for a few months or even years (Simon *et al*., 2015). Although no clear evidence exists to explain the pathogenesis of persistent symptoms following infection, two hypotheses have been proposed: (a) That viral and/or antigenic debris remains in the tissues of joints and muscles. Unfortunately, to date, the virus has not been isolated from such tissue. However, CHIKV proteins have been found in macrophages and muscle cell tissue of relapsing CHIKV patients, supporting the notion that there may be low-grade replication of the virus or non-replicative viral debris present. (b) That infection triggers a persistent immune response. Studies are underway with mouse models to determine whether, and in what way, immunological mechanisms might be altered in patients with persistent symptoms (Burt *et al.*, 2017).

2.7.10. Control and prevention of chikungunya virus

The widely and effective method of prevention of CHIKV is to prevent mosquito bites. The mosquitoes bite during the day and less frequently during the night. Hence, using insect repellent, wearing long-sleeved shirts and pants, treating clothing and gear, and take steps to control mosquitoes indoors and outdoors is advisable (CDC, 2020c).

3. STATEMENT OF THE PROBLEMS, RESEARCH QUESTIONS, AND OBJECTIVES

3.1. Statement of the Problem

In Ethiopia, there are limited recent information in general and specifically no information in the Gambella Region, regarding the circulation of arboviruses, and the associated risk predictors for human and livestock exposure.

In many cases, most arboviral infections remain undiagnosed or misdiagnosed as malaria, typhoid, dysentery, or bacterial meningitis due to the nonspecific nature of the clinical signs/symptoms and the lack of readily available laboratory testing facilities. Hence, due to many factors, arboviruses remain under-recognized and underreported in many parts of the world, particularly in the developing world such as Africa including Ethiopia.

Gambella region is a region in Southwest Ethiopia where vector-borne diseases such as malaria and trypanosomiasis are endemic. Hence, arboviruses infections might have a high chance of misdiagnosis that subsequently would lead to underestimating the true burden of the cases both in humans and in animals.

The rationale of selecting the Gambella region as the study site for this study relied on the following main facts. First, many arboviral diseases have been reported from neighboring countries including South Sudan, Sudan, and Kenya, where the chance of introducing these arboviruses could be high due to the free movement of people, animals, and wildlife across the border together with the migration of people. Second, the ecological suitability for vector reproduction, multiplication, and growing contributes to the survival and transmission of the viruses among various hosts in the area.

Currently, due to climate changes and global warming the growing impact of arboviruses on humans, livestock, and wildlife in many parts of the world is increasing. Knowing the current arbovirus epidemiology particularly in remote areas such as Gambella with pastoralists and smallholder livestock systems would contribute to the national strategic disease control and

prevention system and significantly improve timely response to the challenges of emerging arbovirus in Ethiopia.

In Ethiopia, apart from few outbreak reports of dengue fever and yellow fever the true prevalence of arboviruses circulation remained unknown and diagnosis of febrile illnesses remained a challenge until now. Some of the undiagnosed febrile illnesses in health facilities can be attributed to arboviral infections. The impact and burden of arboviral infection are relating to morbidity and mortality, lead to losses in labor hours, production, and reduction, and reduced economic activities; hence, it was important to study the epidemiology of arbovirus diseases in Ethiopia. Applying this kind of multidisciplinary one health approach in surveillance of arboviruses at humans and livestock interface would help in responding to the emerging challenges in the affected region and ultimately contribute to control and prevention the diseases both in the human and animal population.

3.2. Research Questions

Based on the stated problems this study was expected to address the following main research questions:

- Do the RVFV and WNV circulates in cattle in the selected districts of Gambella Region?
- Do the YFV, CHIKV, and ZIKV circulates in humans in the selected districts of Gambella Region?
- Do the community have awareness about the arboviruses in the selected distrcits if Gambella Region?

3.3. Study Hypothesis

The working hypothesis of this study relied on the postulate, which states, “YFV, CHIKV, and ZIKV infections occurred in humans while RVFV and WNV infections in cattle in selected districts of Gambella Region”.

3.4. Research Objectives

3.4.1. General Objective

The general objective of this study was

- To investigate the sero-epidemiology of some selected arboviruses in cattle and humans in the Gambella Regional State, southwest Ethiopia and assess the level of awareness of the community on arboviruses in the study area**.**

3.4.2. Specific objectives

- To investigate the seroprevalence of RVFV, and WNV infections in cattle in the selected districts of Gambella Regional State, southwest Ethiopia
- To investigate the seroprevalence of YFV, CHIKV, and ZIKV infections in humans in the selected districts of Gambella Regional State, southwest Ethiopia
- To assess the level of awareness of the community about some arboviruses in the selected districts of Gambella Regional State, southwest Ethiopia

4. MATERIALS AND METHODS

4.1. Study Area and Population

Ethiopia is one of the populated federated land-locked countries located in East Africa with neighboring countries namely Kenya, Somalia, Djibouti, Eretria, Sudan, and South Sudan. Administratively Ethiopia is divided into 10 regional states listed as Gambella, Southern Nations Nationalities and Peoples (SNNPR), Oromia, Benshangul Gumuz, Somalia, Afar, Tigray, Amhara, Harari, and Sidama and two city administrations namely Addis Ababa and Dire Dawa (Country of Origin Information, 2020). The federal government is established jointly with these regions and city administrations. The regional government is composed of zones, where the zones are classified into districts (woreda), then into sub-districts (Kebeles) with an average population density of 5000 (Deribe, 2015).

In Ethiopia, the administrative clusters obtain health services using three levels namely primary, secondary and tertiary. The primary health service system is designed to deliver health service at kebele (sub-district) for the rural community that includes five health posts (each for 3000-5000), health centers (serving 15000-25000), and primary hospital (60,000-100,000). In cities and urban settings, the health delivery system is organized with health centers as an initial point serving 40,000 populations. General hospitals that serve from 1-1.5 million populations are included in the secondary level health care delivery system. Tertiary hospitals serve from 3.5 to 5 million people with the maximum catching ability(WHO, 2017a). In the country, there is one federal-level ministry that administers the health system at the top. Every regional state and the city administration has its regional health bureau and also each zone has its health department. Woreda (districts have their own health offices accountable for the district administration council. The district offices have the only technical link between the zonal health bureau and zonal health departments. The public health care operations are governed or supervised by the district health offices, where mainly engaged in planning, monitoring, implementations, and evaluations of the sub-districts health activities under its administration (Wakabi, 2008).

The Gambella Region is located at 7^0 05'to 8^0 17'N latitude and 33^0 00'to $35^0$21' E longitudes in Western Ethiopia. It shares boundaries with the South Sudan Republic in the west, with Sheka zone of Southern Nation and Nationality Peoples Region /SNNPR/ in the northeast, with Maji zone/SNNPR/ in the east, with Illubabor zone /Oromiya Region/ in the north. The Region shows varied topographic features, which influence the vegetation cover, the soil type, and the climatic conditions. The elevation of the region ranges from 1,000 to 2,000 meters above mean sea level in the east, to 500–900 meters in the center, and 300–500 meters in the west (the location of the current study site) (Woube, 1999). It gently slopes to the west, while its eastern part consisted of a high plateau, mountain peaks, and rugged terrain.

According to the Woody Biomass Inventory and Strategic Planning Project (2001), the land coverage of the region falls into three broad ecological-vegetation zones: (i) The main escarpment largely covered with high forest; (ii) foothills and plains covered with lowland forest and savanna woodland; And (iii) to the west extensive grassland plains and seasonally flooded swamps. In addition to the flood, the region is one of the Ethiopian regions, which is rich in water resources. Some of the major rivers include the Baro, Akobo, Alwero, and Gillo (Sewonet, 2002). More than 7.7 % of the total surface area of the region is wetland (open water, perennial and seasonal swam/marsh). The total area of the region is 3,203,280 Km^2 (Woody Biomass Inventory and Strategic Planning Project, 2001).

The annual rainfall of the region is 900mm-1500mm. While it reaches up to 1900-2100mm as the elevation increased to 2000 m. a. s. l. During the wet season (from May to October) 80-90 % of the total rainfall occurs in the region and December, January, and February are considered the dry season. The relative air humidity increases during the wet season (70-80 %) and abruptly decreases in the dry season (43-60 %). Sometimes the maximum daily relative air humidity during the wet season reaches 100 %, while the minimum daily relative air humidity reaches 9% in the dry season. The total annual surface water evaporation value in the region has been estimated to reach 1612 mm; the maximum value (212 mm) occurs in March and the minimum (80 mm) being in July daily. The mean annual temperature of the region varies from 17.3^0c in the mountains to 28.3^0c in the plain. On the other hand, the mean monthly temperature ranges from 27^0c - 33^0c. The absolute maximum temperature reaches 45^0C in mid-March and a minimum of 10.3^0C in December.

Based on the 2021 regional health bureau projection human population of the region are 497,591 of whom 258,747 are men and 238,584 are women (Gambella Regional Health Bureau, 2021).

The Gambella regional livestock and fishery report of the 2021 population estimation of the region as cattle 640,500, sheep 229, 897, goat 268, 402. Equine 12, 749, poultry 1, 212, 771(Gambella Regional Livestock and Fishery Bureau, 2021).

Administratively, the Gambella region is classified into three zones (Agnwa, Nuer, and Majeng), one special district (Itang special), and one city administration (Gambella town). The zones are further classified into 12 districts (Abobo, Gog, Dimma, and Gambella Zuriya district from Agnwaa zone, and six from Nuer Zone namely Jor, Lare, Wantowa, Akobo, Mekuay, and Jikaw; the remaining 2 districts from Majeng zone namely Godere, and Mengesh). In all the districts including the city administrations, there are 247 kebeles (smaller administrative units/sub-districts) where the districts are part of the zones.

Based on the regional health bureau report Lare and Itang special districts with a human population of 52,270 (27,181 males and 25,089 females) and 57,397 (29,272 males and 28, 125 females) respectively(Gambella Regional Health Bureau, 2021). Moreover, Lare district has a total population of 88, 305 cattle, 24,376 sheep, 27,765 goats, 13 equines, and 145,876 poultry, on the other hand, Itang special district has 88,040 cattle, 34,867 sheep, 40,0893 goats, 345 equines, and 150,213 poultry(Gambella Regional Livestock and Fishery Bureau, 2021).

Moreover, the districts are hosting many refugees from South Sudan and also migratory pastoralists from South and North Sudan (commonly called “Fallata” or “Fulani”) in search of pasture and water for their livestock (Miller *et al*.,2005). The indigenous pastoralists also travel far from their villages to the adjacent territories of South Sudan in search of pasture. Indigenous pastoralists also have a tradition of presenting and accepting cattle to or from the South Sudan community during marriage ceremonies, which have the potential to introduce diseases in the area. In addition, the movement of people from outbreak areas of South Sudan to the present area is another potential risk factor of disease introduction (Figure 14).

This study was conducted in the Lare and Itang special districts, Gambella Regional State; southwest Ethiopia located 766km from Addis Ababa, the capital city of Ethiopia (figure 14).

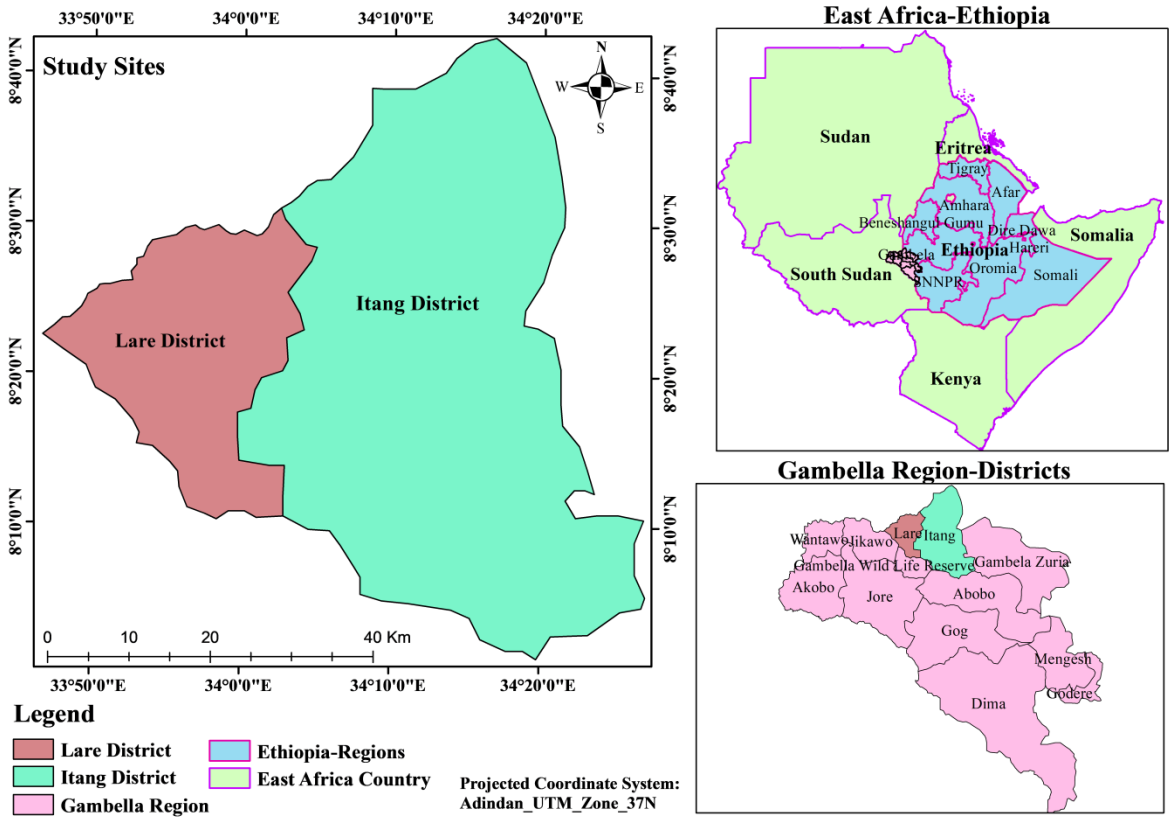


Figure 14. Map of the study site (Lare district and Etang special district on the right side of the map).

Lifestyle of the people and livestock production system

In this region, the types of agricultural activities fall on ethnic groups and territorial partitions. The economy of the western side of highland areas mainly depends on cereal production. The agro-pastoral communities of the region owned a large proportion of livestock and use them mainly in subsistence production style, whereas other groups of people practice husbandry of small livestock numbers.

In the region, there are two main types of livestock production systems; namely extensive pastoral (purely pastoral) systems and mixed crop-livestock (agro-pastoral) systems. The extensive pastoral system is more common in certain groups of ethnic groups and territorial grazing areas of the region. Livestock compositions, grazing management patterns, and herd movement systems are largely determined by seasonal patterns of flooding and flood retreat areas of major rivers. However, the latter system is mainly confined to highland areas above 1500m. a. s. l.(BoFED, 2008).

These two livestock production systems are clearly distinguished by ownership patterns with the relatively large herd sizes in the Nuer agro-pastoral system and the small sizes of the enset-cereal system with no livestock in the Anywa and Mejeng cereal systems. The small-scale subsistence nature of the agricultural system coupled with the frequent droughts and flood crises has been contributing to the low performance and growth of the sector. Nevertheless, the contribution of fisheries to food security is significant in the area (BoFED, 2008).

There are several livelihood activities that households engaged in daily, including crop production, livestock production, crop and livestock production, beekeeping, fishing, hunting, gold mining, and petty trading (BoFED, 2008).

Regarding the status of human and animal health facilities, Gambella Regional state has 1 referral hospital and 28 functional health centers out of 32 health centers. The statistics reflect there should be an intervention in the facilities and human power in the area. Besides, there are three regional hospitals are under construction (WHO, 2014).

There are veterinary clinics and veterinary health professionals at the woreda level. Based on the observational assessment, most of the clinics are not functional; rather they provide vaccination services programs of selected animal diseases not for more than two times per year. A report by Gambella Peoples Regional State Development GAP Assessment Report (2010) showed that the region has animal health facilities that include: three veterinary clinics (25%), out of 12 required as the approved structure, 8 veterinary posts (3.5%), out of

229 required, 12 animal crush (5.2%), and 1 veterinary laboratory (100%) (Gambella Peoples Regional State Development GAP Assessment Report, 2010).

In the area, the genetic distinction of the local animal breeds plays a greater role in disease prevention. Despite the genetic advantages, livestock disease is the main constraint of the production system. Based on previous survey and interview study results where no confirmation was made, previous gap assessments and surveys recorded cattle diseases such as trypanosomiasis, pasteurellosis, contagious bovine pleuropneumonia (CBPP), anthrax, and blackleg. In cattle, sheep, and goats, foot and mouth diseases (FMD), trypanosomiasis, contagious Caprine Pleuropneumonia (CCPP), and internal and external parasites were also mentioned in the survey study. In equines the strangle, tetanus, internal and external parasites were also documented (CSA, 2007; Gambella Peoples Regional State Development GAP Assessment Report, 2010). Common diseases of the human in Gambella include malaria, HIV, tuberculosis (Asebe *et al*., 2015; Sahle *et al*., 2017); Lymphatic filariasis (Shiferaw *et al*., 2012); and many vector-borne diseases such as malaria where it is known as one of the endemic diseases of the area commonly known (Gebre and Negash, 2017).

4.2. Study Design

The study was a cross-sectional type conducted from October 2017 to June 2019 across the selected districts to investigate the seroprevalence of RVFV and WNV infections in cattle, YFV, CHIKV, and ZIKV infections in humans, and seroprevalence of infections in cattle and to assess the community knowledge about arboviruses qualitatively.

4.2.1. Sampling technique

Simple random sampling technique endorsed in this study for community based seroprevalence study, herd level sampling technique for cattle seroprevalnece study and purposeful in site selection.

4.2.2. Data sources

The data for this study was gathered from randomly selected cattle, voluntarily consented community members, and focal field group discussions of the districts.

4.2.3. Sampling frame/population

The study sampling frames were the human, and cattle population of the Gambella Regional States of the selected districts. The participants in the group discussion were selected based on the inclusion criteria, the set of participants were purposefully arranged to include; males, females, young, and adults.

4.3. Sample Size Estimation

4.3.1. ***Sample size estimation for cattle seroprevalence of arbovirus study***

Sample size estimation for cattle

The sample size for cattle in this study was determined using the formula

$$N = \frac{Z^2 \frac{\alpha}{2}\ p(1-p)}{d^2}$$

where Z = 1.96 corresponding value to the desired 95% confidence level, *p* is an estimate of the expected prevalence of infection, and *d* is the margin of error in the estimate(Thrustfield, 2007). In designing this study, the reference was taken to estimate the *p*-value from Southwestern Uganda with an RVFV prevalence of 27%. (Nyakarahuka *et al*., 2018). It has been added 20% to account for the non-response of cattle owners about cattle during sampling, questioning, and dropping of aggressive animals. These assumptions resulted in a minimum sample size of 368 cattle. Animals were included in the study groups when the owners provide an informed/oral consent when animals are in the state for blood sample taking, owner's consent to give brief information about the animal, and age ≥1year. Similarly, animals were excluded from the study in the absence of the mentioned criteria including recently purchased animals from a neighboring country and clinically ill animals.

4.3.2. Sample size determination for community-based human seroprevalence study

The community-based human sample size for the seroprevalence initially was calculated as 238 individuals by taking a study reference conducted in Kenya by Ochieng *et al*. (2015) with a prevalence of arthropod-borne virus infection as 19.2% that was conducted serologically.

$$N = \frac{1.96^2 0.192(1-0.192)}{0.05^2} = 238$$

During the study time, it was only able to obtain 150 volunteer community members. Hence, all community members in the two districts who are residents in the kebele for at least six months, age ≥18 years, and who can provide a written or oral consent to participate were included in the sampling procedure during the study durations. Those who refused to sign or give written consent, guest or foreigner, a person with other illnesses, psychiatric problems were excluded from the study.

4.3.3. Sample size estimation for community and health workers awareness about arboviruses

A qualitative study was implemented to assess the awareness of the community and health workers about arboviruses in the selected kebeles of the two districts. The kebeles where the community-based group discussion (FGD) were conducted in Lare (Kuerlang, Dor Kor mach, Bilimkun, and NipNip) and Itang special districts (Wat Gach, Waar, Dorong, Pulkod, and Achwa) while, two discussions with the health workers in Itang health center and Kuergang health centers. The sample size for this qualitative study was estimated based on the manageable recommended number from 4 to 12 participants per group (Kitzinger, 1995; Krueger & Casey, 2009; Morgan, 1997). With these assumptions, a minimum of 8 and a maximum of 12 participants per group were enrolled for this qualitative study from each kebele(smallest administrative units). In this study individuals aged above 18 years, residing in the selected kebeles, and volunteer to participate in the study were included. Those with obvious psychiatric, people from the refuge and guests for the area, and unable to provide consent were not included in the group discussion.

4.3.4. Data collection for seroprevalence study of RVFV and WNV infections in cattle

The blood sample was collected from the Lare district because of the immediate adjacent to South Sudan and the nature of the livestock production system, movement, and trading system. Besides, Lare is the main entrance route for cattle from South Sudan. In this cattle seroprevalence study, four pastoral sub-districts or "kebeles" (the lowest government administrative structure in Ethiopia) were selected for the study. These kebeles were Pal Bol, Bilimkun/Pagag, Kechi, and Tandor. Each kebele selection was based on the cattle population, proximity to South Sudan, and their frequent contact with the pastoralists from North and South Sudan, common grazing, and watering locations. Blood has been collected from both sexes with a minimum age of one year and above.

In the course of blood collection from cattle, information about herds, age, sex, and parity was collected which are used as explanatory parameters. The history of mass abortion and the history of mass death of young animals together with other signs such as excess salivation, and loss of appetite, and diarrhea were used to investigate clinical signs mainly about the RVF disease (Appendix 1).

4.3.5. Data collection for seroprevalence study of YFV, CHIKV, and ZIKV infections in humans

In this seroprevalence study, blood was collected from community members with criteria of 1) Ages above 18 years and volunteered to participate 2) at least who lived in the area for six months and above. On the other hand community members were excluded from the study when 1) Pregnant women, 2) Sick individuals, 3) Refugees and visitors to the area. Before conducting the data collection process, the discussion was made about the location of the refuge campuses, community movements with bordering territories and the adjacent sub-districts, their proximity, and other security issues with the local administrate. The other criteria to select the sub-districts were centering the ecology of the area such as forest, water bodies, and the population densities of the area. In this human seroprevalence, data including socio-demographic characteristics, duration of stay in the study area, history of residence or

travel to other countries/areas outside Gambella, history of a bite by *Ae*. spp mosquitoes, and history of vaccination against YF were also collected using a semi-structured questionnaire (appendix 2).

4.3.6. Data collection for community awareness

The qualitative information generated from the focal group discussion was narrowly focused on a semi-guided checklist. This discussion focused on a particular topic organized for research purposes. The data collection process was guided, monitored, and all information obtained during the process was recorded. All the group discussants were given equal opportunity and the idea of every discussant was respected and recorded. During group discussion, the participants' residency district, sex, age, occupation, education label, marital status was also taken. The discussion was mainly focused on selected topics (appendix 3).

In the selected kebeles, 11 FGDs (five with women and 6 with men) were conducted. The FGDs age over 18 years were enrolled, and the discussion with men and women was made separately in respected sites. The participants of the FGDs were recruited upon their agreement to participate in the study and carried out with the help of the Kebele administrator/chairperson of the respected villages.

To ensure consistency in data collection across FGDs, a checklist consisting of points to be discussed was prepared. The contents of the checklist include major public health problems in the area, sources of the diseases, the presence of *Anopheles* and *Aedes* mosquitoes (appendix 4) (supported with pictures Fig 15 a, b and c) (*Anopheles* mosquito stock image, 2014; Shutterstock, 2017). Issues including mosquito biting time and place, type of diseases the mosquitoes transmit about the vectors were discussed. The checklist also addressed whether they have seen/encountered YF like disease (supported with a picture of a patient with yellowing of the eyes due to YF, and other explanations by showing different body parts like head for headache, bleeding of eye, nose, and mouth, back pain, and muscle pain for other mosquito-borne viral diseases) (appendix 5). Lastly, participants also forwarded the prevention of mosquito biting. In FGDs, the mosquitoes were discussed at the genera level where species levels were not studied and known in the area.

One FGD was conducted in each district with the health workers that focused on details of major health problems encountered in the area, common mosquito-borne viral diseases such as YF, and RVF and the like, diagnosis and treatment of febrile patients, and their recommendations on the management of unknown febrile cases.

The health centers were selected based on their location and health workers were selected purposefully based on their service years, duration of stay in the study areas, and their qualification. The FGD discussion was moderated by the research team and trained health workers and recorded using a voice recorder as well as notes. The discussion session was administered using a checklist containing various topics and mediated through a translator who are trained and who can communicate the respective local languages and the Amharic language for better exploring of the information. The facilitator speaks in Amharic while the translator mediates in both the local languages (Anywa and Nuer) and commences an immediate translation for the note-making person and audio recording device simultaneously.

4.3.7. Serological laboratory examinations for RVFV and WNV in cattle

In this study whole blood was the source of the serum samples. Based on the OIE guideline, the blood sample 5ml for cattle was collected separately from each study participant with a sterile vacutainer. The vacutainer is used also for serum separation in addition to the collection. This test tube has a special gel that separates blood cells from serum as well as particles to cause blood to clot quickly. To increase the amount of serum and maximum separation the test tubes were centrifuged at approximately 15 minutes at 3400 rotations per minutes and the serum was separated and stored at -20^0c until screened for antibody (IgG) against WNV, and RVFV using an indirect ELISA assay (Abbexa Ltd, Cambridge UK)(Abbexa Ltd, 2019) described in detail by the manufacturers.

All vacutainer and Nunc test tubes (assembled with a screw-on top for tightens closing) were labelled separately with a unique code referring to each sample collected. Samples were stored at 4-6 ^{0}C in the refrigerator at Gambella Regional hospital and Gambella University until shipment to Addis Ababa. During shipping the serum samples, a marina cooler box with ice-frozen bricks was used to keep the cold chain, and samples were shipped to Addis Ababa University Institute of Pathobiology microbiology laboratory. The serum samples were

stored at -20^{0}C packed in cryovial rack box until screened for antibody (IgG) against the RVFV and WNV.

The seroepidemiological analysis of these viruses were using the detection of IgG antibodies against RVFV, and WNV was conducted based on the manufacturer's protocol ready for each individual(Abbexa Ltd, 2019).

In brief, a 96 well plate has been pre-coated with the target antigen of RVFV/WNV-IgG. Controls or test samples are added to the appropriate wells and incubated. Free components are washed away with wash buffer. HRP conjugated detection reagents are used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colour product that changes to yellow after adding an acidic stop solution. The intensity of the color yellow is proportional to the RVFV-IgG amount bound on the plate. The optical density (OD) absorbance is measured spectrophotometrically at 450nm in a microplate reader, and the presence of RVFV/WNV-IgG can be determined. The laboratory result calculation was made using the mean absorbance of the positive control that should be ≥1.00, mean absorbance of the negative control that should be ≤0.10, with the cut-off value=Negative control + 0.15. The interpretation of the results of each plate has been determined as valid when the positive control value is ≥ 1.00 and the negative control value is ≤ 0.10 if not the test has been considered invalid. When the OD. of samples <cut off, the test samples were considered negative, while OD of samples ≥ cut off value, the test samples were considered positive.

The diagnostic sensitivity and specificity have not been determined for these specific kits where they are only ready for research purposes. All the tests were conducted in duplicates. The results were read at an OD of 450 nm using a 96-well ELISA plate reader (Multiskan™ FC Microplate Photometer) and interpreted as positive or negative based on the manufacturer's recommended cut-off values. Test validity was verified according to the manufacturer's manual when the mean OD of the positive control was ≥1 and when the mean OD of the negative control was ≤0.15. The cut-off value for each plate was calculated independently calculated where the cut-off value = negative control +0.15. Hence, any sample value greater than or equal to this value labelled as positive, and any value less than the cut-off value is scored as negative (appendix 6-7).

4.3.8. Serological laboratory examinations for YFV, CHIKV, and ZIKV in humans

Based on the WHO guideline, the blood sample amounted to 3 ml venous blood for humans was collected separately from each study participant with a sterile vacutainer tube. The vacutainer is used also for serum separation in addition to the collection. Similar to the cattle technique the test tube has a special gel that separates blood cells from serum as well as particles to cause blood to clot quickly. To increase the amount of serum and maximum separation the test tubes were centrifuged at approximately 15 minutes at 3400 rotations per minutes and the serum was separated and stored at -20^0c until screened for antibody (IgG) against YFV, and CHIKV using an indirect ELISA assay (Abbexa Ltd, Cambridge UK)(Abbexa Ltd, 2019) described in detail by the manufacturers. The ZIKV also screened using the same serum source with blocking of binding (BOB) assay (Vir Biotechnology inc, 2020).

All vacutainer and Nunc test tubes for human samples were labeled separately with a unique code referring to the sample collected for each. Samples were stored at 4-6 ^{0}C in the refrigerator at Gambella Regional hospital until shipment to Addis Ababa. During shipping the serum samples, a marina cooler box with ice-frozen bricks was used to keep the cold chain, and samples were shipped to Addis Ababa University Institute of Pathobiology microbiology laboratory. The serum samples were stored using the Nunc test tubes assembled with a screw-on top for tightens closing and stored at -20^0c packed in cryovial rack box until screened for antibody (IgG) against the YFV, CHIKV, and ZIKV.

In this seroepidemiological analysis the detection of IgG antibody against YFV, and CHIKV was conducted based on the manufacturer's protocol ready for each individual(Abbexa Ltd, 2019). The human serum was also screened for IgG antibody against ZIKV infection using ZIKV NS1 BOB assay as previously described (Vir Biotechnology inc, 2020).

In brief the procedure of YF-IgG/CHIKV-IgG, a 96 well plate has been pre-coated with the target antigen. Controls or test samples were added to the appropriate wells and incubated. Free components were washed away with wash buffer. A horseradish peroxidase (HRP) conjugated detection reagents were used to visualize enzymatic reactions. The Tetramethylbenzidine (TMB) is catalyzed by HRP to produce a blue color product that

changes to yellow after adding the acidic stop solution. The intensity of the yellow color is proportional to the YFV-IgG/CHIKV-IgG amount bound on the plate. The optical density (OD) absorbance is measured spectrophotometrically at 450nm in a microplate reader, and the presence of YFV-IgG/ CHIKV-IgG can be determined for the laboratory (appendixes 8-9).

The IgG antibody detection against ZIKV infection using the ZIKV NS1 BOB assay which was previously developed by Balmaseda *et al.*(2017) in Nicaragua is highly specific for ZIKV with minimal to no cross-reactivity to other flaviviruses (Balmaseda *et al*., 2017). In brief, 96 well plates were coated overnight with recombinant purified 1µg/ml ZIKV NS1 Uganda strain (Native antigen) protein and blocked with Phosphate-buffered saline (PBS) 1% Bovine serum albumin (BSA) for 1 h. Plates were then incubated with 1:10 diluted serum from each study participant for 1 hour and then HRP labeled anti-ZIKV NS1 (mAB ZKA35, Absolute antibody) was diluted 1:5000 in PBS with 1% BSA and the mixture was added to each well and incubated for another 15 min. Plates were then washed and TMB substrate (Sigma) was added to each well and incubated for 5-6 min in the dark and the reaction was stopped with 2N H_2SO_4 or 1N HCL. Plates were then read on a plate reader (Multiskan™ FC Microplate Photometer) at an absorbance of 450 nm. Non-conjugated ZKA35 rIgG1 (1:200 diluted) was added as positive control and Normal-human serum (NHS) was added as a negative control (1:10 diluted). The percentage of ZKA35-HRP binding inhibition was calculated and is described in detail by Balmaseda *et al.*(2017).

The concentration of ZKA35-HRP to be used in the BOB assay was determined corresponding to 70% of the maximal OD (450 nm) level by interpolating a curve fitted with a 4-parameter nonlinear regression. A starting dilution at 1:10 and then 1:3 serial dilution of 12 points in assay diluent was performed using 50 µl ZKA35-HRP/well (appendix 10). Test validity was verified according to the manufacturer's manual when the mean OD of the positive control was ≥1 and when the mean OD of the negative control was ≤0.15. The cut-off value for each plate was calculated independently calculated where the cut-off value = negative control +0.15. Hence, any sample value greater than or equal to this value labelled as positive, and any value less than the cut-off value is scored as negative.

4.4. Data Management and Statistical Analysis

Data and sample collection instruments

This study was used stationary, serological testing kits of various arboviruses, iceboxes, blood collecting vacutainer tubes, syringes, needles, disinfectants, cotton, pictures..etc. The survey study was handled using a prepared semi-guided checklist and audio recording device for the focus group discussants.

Methods of Data Analysis

All the audio-recorded information gathered from FGDs was transcribed verbatim and translated from local languages Nuer and Anywa into English. The data were analyzed using manual counting of the FGDs discussant's response in each group as a thematic form.

After the completion of data collection, cleaning, editing, and coding data were entered into a computer using Epi-Data Software v.3.1. and analyzed using STATA version 13.0. In continuous and frequency of categorical variables, the mean and standard deviations were used as a descriptive form of the analysis.

The seroprevalence of CHIKV, RVFV, YFV, WNV, and CHIKV elicited towards IgG was calculated by dividing the total positives numbers of each virus with the total number of samples tested. This seroprevalence indicates the apparent prevalence. Univariable logistic regression was used to assess the crude association between the seropositivity of IgG antibody and the hypothesized individual potential risk factors such as age, sex, parity, and site, calculated with descriptive and analytical analysis using chi-square ($\chi 2$) test. Multivariable logistic regression analysis was used to assess the effect of each of the independent variables on the outcome variable (seropositivity) after adjusting each independent variable for all other variables and confounding factors. Those variables which were statistically significant in multivariable analysis were considered as final predictors of the dependent variable of interest. Adjusted odds ratio (AOR) with associated confidence interval was used as a measure of the strength of associations between different exposure variables and the outcome variable of interest. Coefficients of all independent variables (predictors/risk factors) included in the final model were reported regardless of their statistical significance. A p-value below 0.05 was considered indicative of a statistically

significant association. All the blood samples were checked using the recommended standard ELISA test kits.

Data Quality Control

Various data control measures were used for data collection in the field to the laboratory investigations level based on laboratory standard operating procedures (SOPs). A similar orientation was given for all data collectors on the objectives of the research. When an error is found during pre-analytical, analytical, and post-analytical processes was found correction measure was made instantly. Including the laboratory examinations, all the investigators checked the process of analytical procedures. Hence, at the end of each day, the information obtained from the focal group discussion of each participant was written, information was interpreted based on the voting system and averages, and corrective measures were taken. Three to five milliliters of blood samples were taken aseptically from humans and animals with specific codes for each sample containing all necessary information on another sheet of paper respectively. All blood samples were processed according to the SOPs of the manufacturers and laboratory system.

4.5. Study Variables

Dependent variables for community-based FGDs

- ✓ The overall knowledge about diseases of public health importance of arboviruses
- ✓ The overall knowledge about diseases transmitted by *Anopheles* and *Aedes* mosquitoes
- ✓ Health workers experience on major public health problems

Dependent variable for human seroprevalence study

- ✓ Sero-positivity of IgG antibody against YFV infection among community members
- ✓ Sero-positivity of IgG antibody against CHIKV infection among community members
- ✓ Sero-positivity of IgG antibody against ZIKV infection among community members

Dependent variable for cattle seroprevalence study

- ✓ Sero-positivity of IgG antibody against RVFV infection among cattle population
- ✓ Sero-positivity of IgG antibody against WNV infection among cattle population

Independent variables for knowledge of community members FGDs studies

- ✓ Age, sex, education, districts, marital status, ethnic groups (for community-based FGDs), and district, qualifications, age, and work experience

Independent variables for human seroprevalence study

- ✓ Sex, age, educational level, occupation, and districts
- ✓ History of vaccination, History of residence/travel outside Gambella, History of working in the forest areas, History of bite by *Aedes* mosquito

Independent variables for animal seroprevance study

- ✓ Age sex, and kebeles (as a demographic factor)
- ✓ Breeding and clinical history (parity, history of abortions, history of mass death in your animals, previous signs and symptoms of excess salivation in sampled animals, previous history of loss of appetite, diarrhea, and excess nausea in sampled animals)

4.6. Ethical Considerations

The Institutional Review Board (IRB) of the Aklilu Lemma Institute of Pathobiology, Addis Ababa University, approved the study protocol for humans. On the other hand, ethical issues of the animal study were cleared and obtained from the Addis Ababa University College of Veterinary Medicine and Agriculture ethical committee. The Ethical clearance of human sample taking includes the transportation of blood samples from the study site until the processing laboratories. The aim of the study was explained to each of the study participants and written consent was obtained from each participant. Blood sample collection was carried out under aseptic conditions by experienced medical laboratory technicians. Besides, working permission was obtained from the Gambella Regional Health Office and Livestock and Fishery Offices, Woreda administration office by holding a letter of support from the Addis Ababa University. For every information gathering and blood sample-taking activity, oral and written consent was obtained from the animal owner. The name of the study participants was not used in the report and unique identification numbers were used for sample identification in the laboratory.

5. RESULTS

5.1. Seroprevalence of RVFV and WNV Infection Study in Cattle

5.1.1. Characteristics of the study cattle and owners' responses on selected clinical signs

A total of 275 (74.7%) female and 93 (25.3%) male cattle were included in the current study. The majorities(60.3%) were in the age groups of 1- 3.5 years, the minimum and maximum age of the sampled cattle population was 1 and 20 years, respectively, and mean age ± SD=3.9+3.23=7.1years. Among 121 cows, the overall mean birth rate was 3.0 calves per cow (standard deviation = 2.2). The number of births per cow ranged from 1 to 14 calves. The animals came from 53 herds with a minimum and a maximum herd size of 7 and 67 animals, respectively, and a mean herd size of 23.2. Just over half (50.9 %) of the herd owners described mass abortion in the area and 43% mentioned the deaths of young animals within their herd within the past three to five years. Among the herd owners, 43.4% mentioned the historical presence of mass death in their animals (Table 1).

Table 1: Characteristics of the study cattle and owners' responses about selected clinical signs

Characteristics	**Response category**	Number	Percent
Sex	Male	93	25.3
	Female	275	74.7
Age group (N=368)	1 - 3.5 years	222	60.3
	+3.5 years	146	39.7
Parity (N=275)	Yes	121	44.0
	No	154	56.0
Study kebele/site (N=368)	Pal Bol	41	11.1
	Kech	152	41.3
	Bilimkun	89	24.2
	Tandor	86	23.4
History of abortion (N=53 herds)	Yes	27	50.9
	No	26	49.1
History of mass death in your animals (N=53 herds)	Yes	23	43.4
	No	30	56.6
Previous signs and symptoms of excess salivation in sampled animals (N=368)	Yes	52	14.1
	No	316	85.9
Previous history of loss of appetite, diarrhea, and excess nausea in sampled animals (N=368 sampled animals)	Yes	96	26.1
	No	272	73.9

5.1.2. Seroprevalence of RVFV infection in cattle

Out of 368 analyzed serum samples, 28 (7.6%) (95% CI: 5.29-10.81%) were positive for IgG antibody to RVFV infection, which was significantly associated with a history of abortion (14.8%; p=0.041) and no IgG positives were found among those having no history of abortion in their herd. However, there was no significant association between IgG positivity and the study kebeles (villages) (Table 2).

Table 2: Associate and risk factors of seropositivity for RVFV infection in cattle in Gambella

Variables	Response group	IgG Sero status of RVFV		Chi-square value	P-value
		Total tested	Positive No. (%)		
Sex	Male	93	9 (9.7)	0.76	0.384
	Female	275	19 (6.9)		
Age group (N=368)	1 - 3.5 years	222	16(7.2)	0.12	0.720
	3.5+ years	146	12(8.2)		
Parity (N=275)	Yes	121	10 (8.3)	0.62	0..432
	No	154	9(5.8)		
Kebele	Pal Bol	41	3 (7.3)	6.62	0.085
	Kech	152	8(5.3)		
	Bilimkun	89	5(5.6)		
	Tandor	86	12 (13.9)		
History of abortion (N=53 herds)	Yes	27	4(14.8)	4.20	0.041
	No	26	0 (0.0)		
History of mass death in your animals (N=53 herds)	Yes	23	3 (13.1)	1.76	0.185
	No	30	1(3.3)		
Previous signs and symptoms of excess salivation in sampled animals (N=368)	Yes	52	6 (11.5)	1.33	0.249
	No	316	22(7.0)		
Previous history of loss of appetite, weakness, diarrhea, and excess nausea in sampled animals (N=368 sampled animals)	Yes	96	6 (6.3)	0.34	0.560
	No	272	22 (8.1)		
All		368	28(7.6)		

5.1.3.Independent predictors of seropositivity for RVFV infection and the associated risk factors

In a multivariable logistic regression analysis where sex, age, parity, sub-districts, and selected clinical signs considered were taken as independent predictors, no significant

association between the RVFV specific IgG antibody and these predictor variables were observed (Table 3).

Table 3: Univariable and Multivariable logistic analysis of risk factors for RVFV seropositivity in Gambella

Characteristics	Response category	**Sero-positive status of RVFV IgG**	
		COR (95%CI)	**AOR (95% CI)**
Sex	Male	0.69(0.30-1.60)	0.79(0.22-1.54)
	Female	1	1
Age group (N=368)	1 - 3.5 years	1	1
	3.5+years	1.15(0.53- 2.51)	1.47(0.32-6.82)
Parity (N=275)	Yes	0.87(0.27-1.75)	0.97(0.19-4.91)
	No	1	1
Kebele	Pal Bol	1	1
	Kech	1.42(0.36- 5.62)	1.48(.36-6.05)
	Bilimkun	1.33(0.30-5.84)	1.40(0.30-6.42)
	Tandor	0.49(0.14-1.83)	0.52(0.13-2.09)
Previous signs and symptoms of excess salivation in sampled animals (N=368)	Yes	0.57(0.22-1.49)	0.45(0.14-1.40)
	No	1	1
Previous history of loss of appetite, weakness, diarrhea, and excess nausea in sampled animals (N=368 sampled animals)	Yes	1.32(0.52-3.36)	1.68(0.54-5.18)
	No	1	1

COR= crude odds ratio, AOR= adjusted odds ratio, CI= confidence interval

5.1.4. Seroprevalence of WNV infection and the associated risk factors in Cattle

Table 4 presents the seroprevalence of WNV infection stratified by different background characteristics. The seroprevalence of WNV specific IgG antibodies was detected in 20 serum samples (5.4%) (95% CI: 3.52-8.28%). No significant associations were observed between the findings of WNV antibody IgG and past-recorded clinical signs. On the other hand, significantly higher proportions of anti-WNV IgG antibodies were observed in serum

samples collected from cattle with a history of abortion as compared to those without a history of abortion (11.1% Vs 3.8%, p= 0.04) (Table 4).

Table 4: Seropositivity for WNV infection in cattle

Characteristics	Response category	IgG Sero status of WNV		Chi-square value	P-value
		Total tested	Positive No. (%)		
Sex	Male	93	5 (5.4)	0.76	0.384
	Female	275	15(5.5)		
Age group (N=368)	1 - 3.5 years	222	14(6.3)	0.12	0.720
	3.5+ years	146	5(9.4)		
Parity (N=275)	Yes	121	5(4.1)	0.62	0..432
	No	154	10(6.5)		
Kebele	Pal Bol	41	3(7.3)	6.62	0.085
	Kech	152	9(5.9)		
	Bilimkun	89	7(7.9)		
	Tandor	86	1(1.2)		
History of abortion (N=53 herds)	Yes	27	3(11.1)	4.20	0.041
	No	26	1 (3.8)		
History of mass death in your animals (N=53 herds)	Yes	23	2 (8.7)	1.76	0.185
	No	30	2(6.7)		
Previous signs and symptoms of excess salivation in sampled animals (N=368)	Yes	52	2(3.9)	1.33	0.249
	No	316	18(5.7)		
Previous history of loss of appetite, weakness, diarrhea and excess nausea in sampled animals (N=368 sampled animals)	Yes	96	5(5.2)	0.34	0.560
	No	272	15(5.5)		
All		368	20(5.4)		

5.1.5. Independent predictors of seropositivity for WNV infection and the associated risk factors

The odds of getting WNV positive IgG in all the variables in the multivariable logistic regression did not result in a statistically significant association with any one of the investigated risk factors (Table 5). In this analysis, odds ratio greater than one observed in males, and those with a history of excess salivation signs and symptoms.

Table 5: Multivariable logistic regression of RVFV seropositivity with independent risk factors in cattle

Characteristics	Response group	**Sero-Positive status of WNV IgG**	
		COR(95%CI)	**AOR (95% CI)**
Sex	Male	1.02(0.36-2.87)	1.21(0.39-3.73)
	Female	1	1
Age group (N=368)	1 - 3.5 years	1	1
	3.5+years	0.64(0.24-1.70)	0.61(0.10-3.84)
Parity (N=275)	Yes	1.5(0.532-4.23)	1.07(0.15-7.61)
	No	1	1
Kebele/sub-distrcits	Pal Bol	1	1
	Kech	1.25(0.32-4.86)	0.13(0.01-1.38)
	Bilimkun	0.93(0.23-3.77)	0.18(0.02-1.44)
	Tandor	6.71(0.68-66.62)	0.14(0.02-1.14)
Previous signs and symptoms of excess salivation in sampled animals (N=368)	Yes	1.51(0.34-6.71)	1.57(0.29-8.50)
	No	1	1
Previous history of loss of appetite, weakness, diarrhea, and excess nausea in sampled animals (N=368 sampled animals)	Yes	1.06(0.375-3.01)	0.86(0.24-3.03)
	No	1	1

COR= crude odds ratio, AOR= adjusted odds ratio, CI= confidence interval

5.2. Seroprevalence of YFV, CHIKV, and ZIKV Infection Study in Human

5.2.1. Socio-demographic characteristics of study participants

A total of 150 study participants (96 males and 54 females, age ranging from 18 to 65 years, mean age ± SD = 35.92 ± 10.99 years) participated in the study. The travel or living /residency history outside Gambella was higher in males (16.7%) than females (7.4%), which indicates males were traveling far from their home area to other places. A higher proportion of participants from the Lare district (15.2%) were traveling or residing outside of Gambella, which might be to South Sudan. The majority of travelers were in the age group of 18-30

years of age (17.5%), which could be explained by various socio-economic activities such as trade, education, or marriage. Travel/Residency and working history in the forest area with the socio-demographic characteristics of all study participants are summarized in Table 6.

Table 6: Study participants' socio-demographic characteristics in the seroprevalence of YFV, CHIKV, and ZIKV Study in human

Variables	**Response category**	**Travel history**		**History of working in the forest**	
		Yes N(%)	**No N(%)**	**Yes N (%)**	**No N(%)**
Sex	Male	16(16.7)	80(83.3)	79(82.3)	17(17.7)
	Female	4(7.4)	50(92.6)	47(87.0)	7(13.0)
Woreda (District)	Itang	15(12.8)	102(87.2)	96(82.1)	21(17.9)
	Lare	5(15.2)	28(84.8)	30(90.9)	3(9.1)
Age (years)	18-30 years	10(17.5)	47(82.5)	48(84.2)	9(15.8)
	31-40 years	5(10.2)	44(89.8)	40(81.6)	9(18.4)
	≥41 years	5(11.4)	39(88.6)	38(86.4)	6(13.6)
Ethnicity	Nuer	15(15.5)	82(84.5)	92(94.8)	5(5.2)
	Agnewak	5(9.4)	48(90.6)	34(64.2)	19(35.8)
Education level	Informal	9(9.7)	84(90.3)	83(89.3)	10(10.7)
	Formal	11(19.3)	46(80.7)	43(75.4)	14(24.6)
Occupation	Pastoralist	8(13.8)	50(86.2)	53(91.4)	5(8.6)
	Agro pastoralist	6(16.7)	30(83.3)	35(97.2)	1(2.8)
	Others	6(10.7)	50(89.3)	38(67.9)	18(32.1)

5.2.2. Study participants travel and residency history within and out of Ethiopia

Among those who have residence/travel history outside Gambella, 16 participants were based in South Sudan, two in Sudan, and two were from other regions within Ethiopia. The migration of people from South Sudan to Ethiopia was elevated during the years 2016/2017 but domestic migration from Gambella to other parts of Ethiopia was low overall (figure 15).

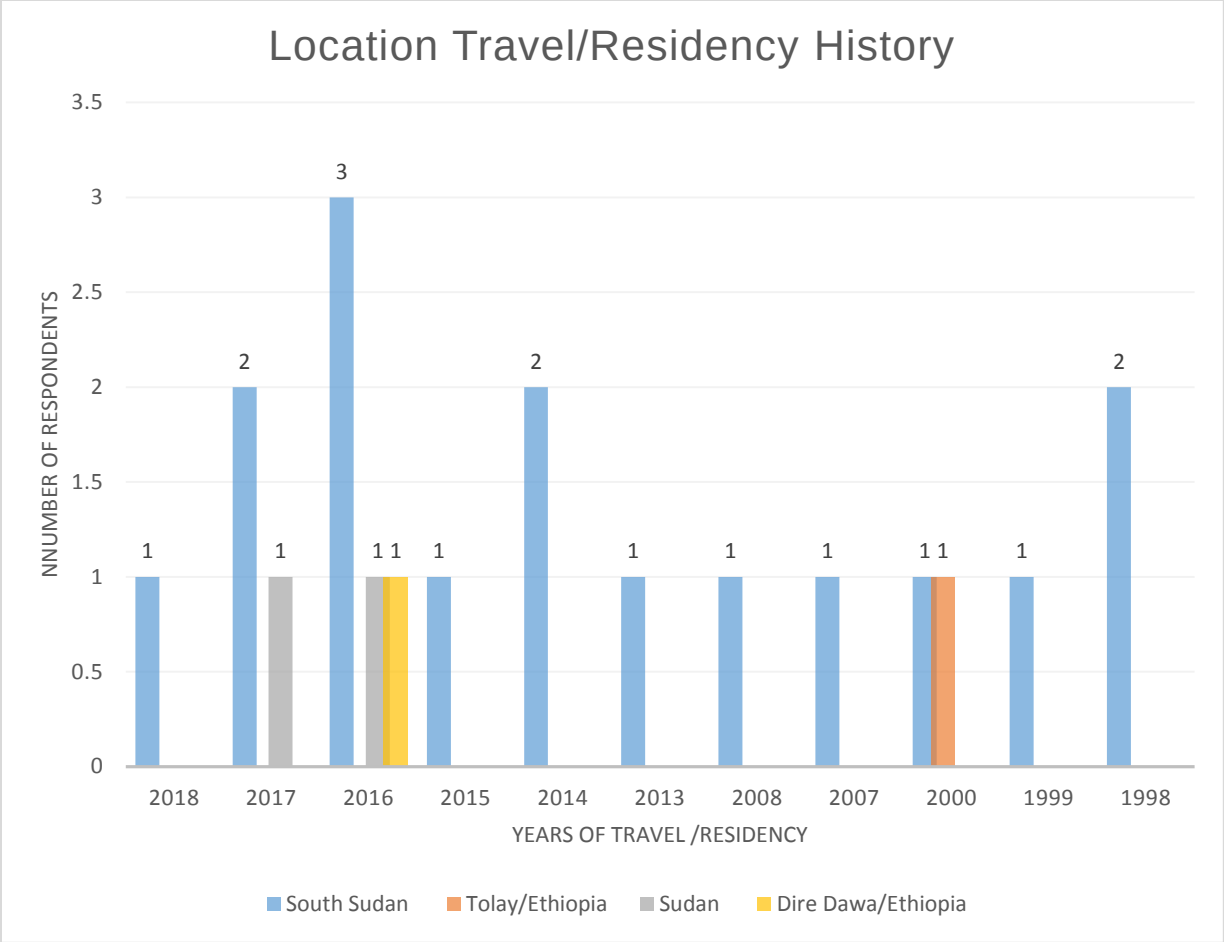


Figure 15. Traveling and residence out of Gambella region among those with history of movement among those who participated in the seroprevalence study of YFV, CHIKV, and ZIKV.

5.2.3. Seroprevalence of YFV, CHIKV, and ZIKV infections in human

In this study, a total of 150 individuals from two districts (33 from Lare and 117 from Itang special district consented and volunteered to participate. Six participants, five from Etang and one from the Lare district were excluded due to sickness. In this serosurvey, 41(27.3%; 95% CI: 20.7, 35.1%), 14 (15.6%, 95% CI: 9.3-24.8%), and 4 (2.9 %; 95% CI: 1.1-7.72%) were positive for IgG to ZIKV, CHIKV, and YFV, respectively, among all study participants (figure 16). Moreover, all YFV IgG+ were found to be CHIKV positive while only one sample was found both of ZIKV and CHIKV IgG+ during screening.

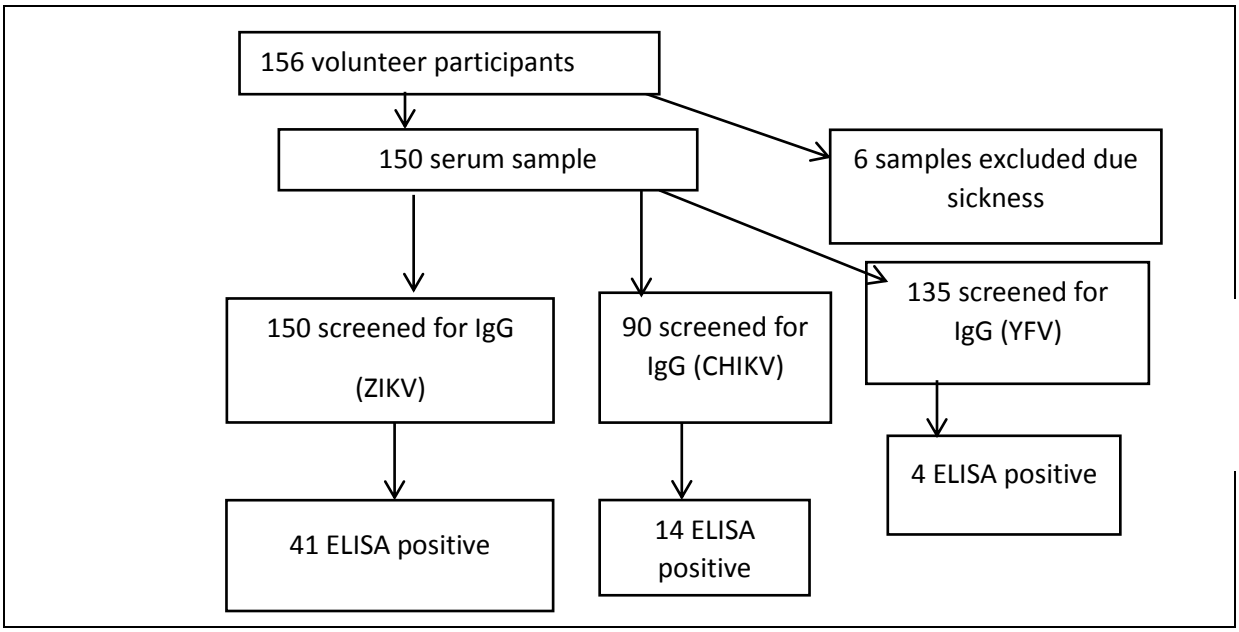


Figure 16. Laboratory work flow chart serum samples screened for ZIKV, CHIKV, and YFV using ELISA Laboratory Investigation

5.2.4. Seroprevalence and independent predictors of seropositivity for YFV infection and the associated risk factors

Out of the 150 serum samples, 135 samples were screened for IgG antibodies to YFV, but only 4 samples (2.9 %; 95% CI: 1.1-7.72%) were found to be YFV IgG+. All the YFV-IgG+ individuals came from the Itang special district; hence, site/district was omitted during logistic regression analysis due to zero outcomes from the Lare district. There were no risk factors significantly associated with the presence of YFV specific IgG antibodies. However, there was a trend that males (3.6%) and individuals in the age group of 31-40 years (5%) were more likely to have YFV specific IgG antibodies compared to the female group (2.0%) or other age categories, respectively (Table 7).

Table 7: Seropositivity for IgG antibody to YFV infection and associated factors in the study participants (N=135)

Variables	Category	YF IgG sero-status		COR (95% CI)	AOR (95% CI)
		Total tested	Positive N (%)		
Sex	Male	84	3 (3.6)	1.85(0.19, 18.30)	1.32(0.10, 17.03)
	Female	51	1(2.0)	Ref	Ref
Age Group (years)	18-30	53	1(1.9)	0.79(0.05, 12.99)	0.77(0.03, 17.64)
	31-40	40	2(5.0)	2.16(0.19, 24.77)	2.29(0.17, 31.61)
	≥41	42	1(2.4)	Ref	Ref
Educational Status	Formal education	51	2(3.9)	1.7(0.23, 12.26)	1.31(0.10, 17.23)
	Informal education	84	2(2.4)	Ref	Ref
Occupation	pastoralist	55	1(1.8)	Ref	Ref
	Agro pastoralist	32	1(3.1)	1.74(0.10,28.84)	1.92(0.11, 35.05)
	Others	48	2(4.2)	2.35(0.21, 26.73)	1.18(0.05,25.41)
History of residence/travel outside Gambella	Yes	17	1 (5.9)	2.4(0.23, 24.45)	3.10(0.21, 44.56)
	No	118	3 (2.5)	Ref	Ref
History of working in the forest areas	Yes	113	3(2.7)	0.57(0.06, 5.77)	0.47(0.03, 7.68)
	No	22	1(4.8)	Ref	Ref
History of Bite by *Aedes* mosquito	Yes	109	3(2.7)	0.54(0.05, 5.44)	0.74(0.04, 12.25)
	No	26	1(3.85)	Ref	Ref

CI (confidence interval), COR (crude odds ratio), and AOR (adjusted odds ratio), Ref (Reference category)

5.2.5. Seroprevalence and independent predictors of seropositivity for CHIKV infection and the associated risk factors

Out of the 150 samples, 90 samples were screened for IgG antibodies against CHIKV infection. Among the screened samples 14 samples (15.6%, 95% CI: 9.3-24.8%) were positive for IgG antibodies against CHIKV. In this logistic regression analysis, site/district was included due to CHIKV-IgG positive was found in both locations, where no zero outcomes present. In the multivariable regression analysis model, a 14-fold lower seropositivity for CHIKV specific IgG was detected among pastoralist participants compared to agro-pastoral (AOR=14.17; CI: 2.30, 87.30). A higher proportion of anti-CHIKV IgG

antibodies was also observed in the age group ≥41. Although it is not statistically significant, higher odds of being CHIKV IgG positive was observed in males, and between the age groups of 31-40 years) (Table 8).

Table 8: Sero-positivity for IgG antibody to CHIKV and associated factors in the study participants (N=90)

Variables	**Category**	**CHIKV IgG sero-status**		**COR (95% CI)**	**AOR (95% CI)**
		Total tested	**Positive** N(%)		
Site	Itang	58	12(20.7)	Ref	Ref
	Lare	32	2(6.3)	0.25(0.05, 1.22)	0.43(0.06,2.82)
Sex	Male	53	12(22.6)	5.12(1.07, 24.46)*	3.78(0.58, 24.52)
	Female	37	2(5.4)	Reference	Reference
Age Group (years)	18-30	36	4(11.1)	0.48(0.12, 1.89)	0.75(0.11, 5.37)
	31-40	25	4(16.0)	0.73 (0.18, 2.95)	2.74(0.47, 16.08)
	≥41	29	6(20.7)	Ref	Ref
Educational Status	Informal education	64	8(12.5)	Ref	Ref
	Formal education	26	6(23.1)	2.1(0.65, 6.80)	2.64(0.36, 19.29)
Occupation	Pastoralist	49	2(4.1)	Ref	Ref
	Agro pastoralist	26	9(34.6)	12.44(2.44,63.47)*	14.17(2.30, 87.30)*
	Others	15	3(20.0)	5.88(0.88, 39.21)	2.20(0.17, 27.18)
History of residence outside Gambella	Yes	10	3(30.0)	2.68(0.60, 11.98)	2.44 (0.33, 17.69)
	No	80	11(13.7)	Ref	Ref
History of working in the forest areas	Yes	82	13(15.8)	1.32(0.14, 11.64)	0.90(0.05, 15.33)
	No	8	1(12.5)	Ref	Ref
History of Bite by *Aedes* mosquito	Yes	89	12(15.2)	0.54(0.10, 2.98)	0.73(0.1, 8.00)
	No	11	2(18.2)	Ref	Ref

CI (confidence interval), COR (crude odds ratio), AOR (adjusted odds ratio), and *(significant at $p<0.05$), Ref (Reference category)

5.2.6. Seroprevalence and independent predictors of seropositivity for ZIKV infection and the associated risk factors

Out of the 150 samples tested for IgG antibody to ZIKV infection, 41 samples (27.3%; 95% CI: 20.7, 35.1%) were found ZIKV IgG+. Multivariable logistic regression analysis showed that being female (AOR=4.8; 95% CI: 1.62, 14.64), being a pastoralist by occupation (AOR=5.1; 95% CI: 1.44, 17.80), and Lare in location showed a significant association with seropositivity for IgG antibody to ZIKV. An increasing trend towards higher ZIKV IgG+ was seen amongst individuals in the age groups 18-30 years, and in those with a history of residence /travel outside of Gambella, and a history of working in the forest areas, nevertheless, none of them reached statistical significance (Table 9).

Table 9: Sero-positivity for IgG antibody to ZIKV and associated factors in the study participants (N=150)

Variables	**Category**	**ZIKV IgG sero-status**			**COR (95% CI)**	**AOR (95% CI)**
		Total tested (%)	**Positive N (%)**	Equivocal N (%)		
Site	Itang	117	15(12.8)	2(1.7)	Ref	Ref
	Lare	33	26(18.8)	0(0.0)	21.8(8.20, 58.23)*	11.5(3.31, 39.81)*
Sex	Male	96	15(15.6)	1(1.0)	Ref	Ref
	Female	54	26(48.2)	1(1.8)	5.0(2.34, 10.65)*	4.8(1.62, 14.63)*
Age Group (years)	18-30	57	23(40.3)	0(0.0)	2.1(0.90,4.8)	1.5(0.39, 5.61)
	31-40	49	11(22.5)	1(2.0)	1.5(0.53, 4.0)	0.80(0.21,3.13)
	≥41	44	7(15.9)	1(2.3)	Ref	Ref
Educational Status	Informal education	93	32(34.4)	1(1.1)	2.59(1.16, 5.76)*	1.0(0.28,3.68)
	Formal education	57	9(15.8)	1(1.8)	Ref	Ref
Occupation	Pastoralist	58	32(55.2)	1(1.7)	8.2(2.78, 24.1)*	5.1(1.44, 17.80)*
	Others	56	5(8.9)	0(0.0)	0.61(0.16, 2.27)	1.5(0.27,8.22)
	Agro pastoralist	36	4(11.1)	1(2.8)	Ref	Ref
History of residence /travel outside Gambella	Yes	20	6(30.0)	0(0.0)	1.10(0.38, 3.0)	1.4(0.29,6.65)
	No	130	35(26.9)	2(1.5)	Ref	Ref
History of working in the forest areas	Yes	126	36 (28.6)	2(1.6)	1.64(0.57, 4.72)	1.15(0.20, 6.49)
	No	24	5(20.8)	0(0.0)	Ref	Ref
History of bite by *Aedese* mosquito	Yes	118	36(30.5)	2(1.7)	5.7(1.28, 25.37)*	1.34(0.21, 8.47)
	No	32	5(15.6)	0(0.0)	Ref	Ref

CI (confidence interval), COR (crude odds ratio), AOR (adjusted odds ratio), and *(significant at $p<0.05$), Ref (Reference category)

5.3. Community awareness and experiences of health workers concerning mosquito-borne viral diseases

Socio-demographic characteristics of the study participants

At a community level, 122 participants (60 men and 62 women, age range 19-73 years, SD± 11.8, average=43.1 years) participated in the FGDs. Most of the study participants (94.6%) were dependent on livestock rearing and with no elementary education (84.4%) (Table 10). A total of 16 health workers (all men, age range 24-36 years, SD±3.5, average=29 years) participated in the FGD (Table 11).

Table 10: Socio-demographic characteristics of participants involved in the community-based FGD

Variables	Number of participants	Percent (%)
District		
Itang	56	45.9
Lare	66	54.1
Gender		
Men	60	49.2
Women	62	50.8
Age category		
19-24 years	5	4.09
25-44 years	56	45.9
45-64 years	55	45.08
≥65 years	6	4.9
Education		
Illiterate	103	84.4
Diploma	1	0.8
Secondary school	18	14.8
Occupation		
Agro pastoralist	7	5.7
Pastoralist	115	94.3
Marital status		
Married	120	98.4
others	2	1.6
Ethnic group		
Nuer	118	96.7
Anuak	4	3.3

Table 11: Socio-demographic characteristics of health workers

Variables	Number of participants	Percent (%)
Health center		
Itang	6	37.5
Lare	10	62.5
Qualification of health workers		
Clinical nurses	12	75.0
Health Officer	2	12.5
Laboratory technician	2	12.5
Age category		
19-24 years	1	6.2
25-44 years	15	37.5
Work experience in the area		
2-5 years	9	56.3
Above 5 years	7	43.8

Community knowledge about diseases of public health importance

When men were prompted about common public health problems, they listed diseases such as malaria, typhoid, TB, hemorrhoid, HIV, and disease caused by Zoster virus locally they call it to spider urine infection (herpes zoster virus infection) in all the groups held. Some of the study participants mentioned clinical signs rather than mentioning the disease by name, thus public health concerns were expressed as clinical syndromes or signs such as fever, diarrhea, headache, stomachache, back and joint pain, sudden death, vomiting, coughing, skin diseases, eye problem, and abortion,

In addition, men participants from Nip Nip kebele stated that unknown diseases characterized by high fever, joint and back pains could be transmitted from animals to humans through the consumption of milk and meat of sick or dead animals. Participants also mentioned the occurrence of similar diseases between animals and humans at a similar time. The discussants requested regular disease prevention programs for their animals as well. During the discussions, they also exhibit their sick animals in this sub-district. The participants also complained about diseases caused by drinking and/or using pond water for cooking food.

Likewise, women mentioned diseases such as malaria, typhoid, skin disease, diarrhea, joint problems and spider disease, bone TB, and fever-related diseases as the common public health problem in the study areas. They also outlined disease signs rather than their names as skin diseases, diarrhea, joint problems, and spider urine infection (herpes zoster virus infection). One woman said, "*There is a disease that we do not know its name or the cause. Its signs are bloody diarrhea, fever, and joint pain. It affects all age groups and both sexes"*.

Community knowledge about diseases transmitted by *Anopheles* and *Aedes* mosquitoes

When shown the pictures (appendix 5), the participants (almost all of the men and women) identified *Anopheles* mosquitoes (locally known as Nyise in Nuer language and Bewo in Agnwak languages*)* as the vector of malaria. They also mentioned that malaria is common during the rainy season and it affects those individuals who frequently work in forest areas. The participants identified malaria by its clinical signs/symptoms such as fever, headache, back and/or joint pain, and weakness. They also mentioned that using bed nets and cloths as a blanket can protect from mosquito bites and thus prevent malaria.

However, there is a misperception in one of the women group participants as some of them suggested, "*When a person is showered with rain, or stayed outside in the rain and come back to home, he/she will be sick of malaria, so rain is also the cause of malaria***"**. One woman also mentioned that malaria could be transmitted from mother to child through breastfeeding.

All women and men recognized *Aedes* mosquitoes (also locally known as forest black-and-white color Nyise/Bewo) (appendix 5). They also indicated that *Aedes* mosquitoes bite both humans and animals during the daytime in the forest area. Nevertheless, the participants expressed that they had no information on the specific disease that can be transmitted by *Aedes* mosquitoes. They said that bites by *Aedes* mostly result in itching. In the groups, very few of the participants mentioned *Aedes* bites can also cause malaria and some other unknown diseases. One old man said, "*We know another disease that results in bleeding through the nose and mouth. It looks like malaria, but it has some differences. The difference is that there is variability in its fatality. Some may die immediately and others stay for a while*". Participants from Lare district, especially those from Pakag, a village on the border of South Sudan, strongly argued that biting by this *Aedes* mosquito can cause malaria-like

disease, which can kill within a few days due to bleeding through the nose/ mouth and/or by changing eyes color to yellowish. They added that the disease has occurred every year since 1991 during the rainy season or after the rains when the population of mosquitoes is abundant regularly. Yellowish eye color incidences are almost mentioned in all the FGDs discussed in both male and female groups. They said that the disease killed more than ten individuals in this area during the August and beginning of September 2017. They thought that 'the disease came from other places like South and North Sudan because of migration of pastoralists and refugees'.

One woman from Itang special district said: *"Some years ago I have seen a person who was sick and his eyes became yellowish".* The woman added, "*That disease is characterized by signs/symptoms, like fever, bleeding through nose and mouth and it is a killer disease which has no treatment".* Another woman from the Lare district said, "A *year ago my six years old son was sick from a disease which caused eyes, palm, and fingers to develop a yellowish color, but he recovered without any treatment within about seven days*". She added, "*I also remember a 12-years old boy from a neighbor who was also sick from a similar disease, but at that time it was said to be malaria and he recovered".*

Health workers experience major public health problems in the area

Health professionals from both Itang and Lare health centers mentioned diseases such as malaria, diarrheal diseases, sexually transmitted diseases, pneumonia, typhoid, malnutrition, TB, HIV, and visceral leishmaniasis as major health problems. However, almost all the participants said that they did not encounter or had information on any disease other than malaria that is transmitted through mosquitos' bites in the area. Occasionally, they may run out of Rapid Diagnostic Test (RDT) or reagents used for malaria diagnosis, and they treat the patient for malaria based on findings of clinical examination. If the patient comes back after three days of taking an anti-malarial drug and has not recovered, they suspect typhoid fever and provide treatment accordingly without laboratory confirmation because of a lack of reagents. If a person treated for malaria and typhoid still suffers from fever and headache or other signs, they test again for the presence of malaria and give treatment for typhoid fever for the second time of the same patient to clear out whether the patient is suffering from the first case or a different one.

They also stated that sometimes they refer such cases to the Regional hospital. When the participants were asked whether they have encountered YF or other mosquito-borne viral disease cases in the area and how they diagnosed them, they said that they did not encounter YF or other mosquito-borne viral disease cases in this area. However, during the discussion, health workers from Etang health center mentioned that they suspected YF- like cases, which caused the death of seven individuals from ten suspected cases in 2016 in a village, called Acula, which is closer to a South Sudan refugee camp. They also stated that they do not have the facility to diagnose and rule out cases of YF or other mosquitoes –borne viral diseases in febrile patients who are negative for malaria or typhoid fever.

6. DISCUSSION

Emerging and re-emerging mosquito-borne viruses such as Yellow fever virus (YFV), Chikungunya virus (CHIKV), Dengue virus, and Zika virus (ZIKV) have become a major public health concern in tropical and subtropical countries (CDC, 2020h; Gould & Higgs, 2009; Lilay *et al*., 2017). Arboviral infections and outbreaks in Ethiopia have been documented since the 1960s, yet the present epidemiological situation in the country remains unknown (Ardoin *et al*., 1976). A limited number of studies on arboviruses in human (Ardoin *et al*., 1976; Ferede *et al*., 2021; Lilay *et al*., 2017; Mengesha Tsegaye *et al*., 2018; Woyessa *et al*., 2013) and livestock (Ibrahim *et al*., 2020) have been carried out in Ethiopia. This seroprevalence study focused on arboviruses in cattle (RVFV, WNV) and human community members (YFV, CHIKV, and ZIKV) in the Gambella Region of southwestern Ethiopia. Many of the previous few studies on arboviruses have been conducted in other regions of Ethiopia and were mainly part of outbreak investigations at that time including dengue fever (DF) and YF (Ardoin *et al*., 1976; Endale *et al*., 2020; Lilay *et al*., 2017; Mengesha Tsegaye *et al*., 2018; Woyessa *et al*., 2013).

In this study, it has been tried to examine the existence of the arboviruses from three perspectives. The first part was conducted to investigate the seroprevalence of RVFV and WNV in cattle and found an indicative result of antibodies against the two viruses in the serum showing the possible circulation of these arboviruses. The second study was conducted to determine the seroprevalence of YF, CHIKV, and ZIKV on the human at the community level, where evidence of the previous circulation of these arboviruses in the area was shown. The third part focused on community-based focus group discussion which was conducted to determine the community and health workers' awareness about arboviruses in the area.

The antibody evidence in both humans and cattle showed the possible future impacts in suitable conditions for viral distribution at an increased level in the area. Indication of these antibodies together with the low level of the community and health workers about the arboviruses clinical manifestations and signs, transmissions, and the presence of vectors are epidemiological indicators that demand detail future examinations for the control and preventions,

Besides, the availability of the wide host range and the free movement of animals (domestic and wild) and human would favor the viruses introduction in the area.

6.1 Seroprevalence of RVFV and WNV Infections in Cattle

A limited number of studies on arboviruses in livestock (Ibrahim *et al*., 2020) have been carried out in Ethiopia. This seroprevalence study in cattle revealed that there were seropositive for RVFV and WNF infection. Antibodies positive to both viruses were found in cattle from all the four sub-district "kebeles". Comparing the anti-IgG of WNV and RVFV in this study, it has been found a high seroprevalence of RVFV than WNV

The seroprevalence of RVFV infection in cattle previously reported in another African country is 6.2% in the eastern region of the Democratic Republic of the Congo (Georges *et al*., 2018) which is similar to the current study. On the other hand, the prevalence observed in this study was lower than a report from the eastern parts of Ethiopia, Somalia region which 17.9 % (Ibrahim *et al*., 2020), where this could be due to high marketing places in Somali for exporting purpose where different animals come together that lead for high infection while not applied in Gambella. It was also observed a lower prevalence compared to other African countries like Cameroon (9.4%)(Zeller *et al*., 1995), Rwanda (16.8%) (Umuhoza *et al*., 2017); Madagascar (25.8%) (Jeanmaire *et al*., 2011) and Mozambique (36.6%) (Lagerqvist *et al*., 2013).

There are no reports on the clinical importance of WNV infection in cattle (UCD, 2012). Cases of WNF in cattle are rarely documented and serologic findings of WNV-specific IgG antibodies have been reported in other countries like Nigeria. In the Nigerian study, WNV IgG-antibody was documented in different animal sera including camels, goats, cattle, and sheep (Olaleye *et al*., 1990) WNV IgG-antibody was also reported in cattle in Senegal (Davoust *et al*., 2016), in Trinidad (Thompson *et al*., 2012), in Turkey (Ozkul *et al*., 2006), and Palestine (Medhat *et al*.,1983). Several other studies in African countries have also reported anti-IgG RVFV and anti-WNV seropositive cattle among apparently healthy animals, (Fafetine *et al*., 2013) including Kenya *(*Labeaud *et al., 2008;* Munyua *et al*.,2010),

Tanzania (Sumaye *et al.*,2013), Mozambique and Senegal (Lagerqvist *et al*., 2013; Lef`evre, 2000; Munyua *et al*., 2010; Zeller *et al*., 1995)

The current study showed RVFV and WNV specific IgG antibodies present in the serum of cattle, that measures a passive infection. Thus, this study cannot confirm recent infection, and the results may show only the exposure rate in the area. There is an observation of high RVFV and WNV seropositivity rates among the age group of 3.5+ years showing that these groups of animals might have been introduced to the heard at least since 2015. However, WNV prevalence seems to have increased with time compared to RVFV because seropositivity in the age groups of 1-3.5 years was higher in the former than in the same age groups in the latter, where relatively higher seropositivity was observed.

The seropositivity and sex of the cattle showed no significant association indicating that both males and females are affected by RVFV and WNV. However, male cattle were more likely affected by WNV infection than females, while the opposite was true for RVFV infection. Further studies are necessary to look at the reason for this result.

Other factors such as age and sampling sites did not significantly affect the occurrences of both viruses. In contrast to the present study, other studies have found a significant association between seropositivity of these viruses and the two factors (age and sex)(Abdel-amir *et al.*, 2019; Nyakarahuka *et al*., 2018; Ould El Mamy *et al*., 2011; Sumaye *et al*., 2013). A study conducted in Madagascar reported a higher prevalence of RVFV in male cattle compared to female cattle (Jeanmaire *et al*., 2011). In regards to the association of age-seropositivity of IgG antibodies in an endemic country, the occurrence of IgG antibodies in older animals generally supports the contention that most animals are exposed to the virus during their lifetimes. Observing IgG+ in younger animals permits to pinpoint the time window of the infection, and how the recent infection occurred or was introduced (Jeanmaire *et al*., 2011; Sumaye *et al*., 2013). Based on the multivariable logistic regression analysis, this study revealed 1.5 times more likely to be seroprevalence of RVFV in the age group of 3.5+ years and a high prevalence of WNV infection in the age group between 1-3.5 years, which is in line with earlier studies showing a high proportion of IgG+ in similar age groups

(Georges *et al*., 2018; Jeanmaire *et al*., 2011; Sumaye *et al*., 2013). Enrolling a larger sample size might reveal a different association of the IgG result with age and sex.

Livestock owners were also interviewed on some selected clinical signs related to RVF infection since the WNF clinical signs in cattle have not been described. Almost half (50.9%) of the owners reported the occurrence of mass abortion in their herds, a finding significantly associated with RVFV IgG+ seropositivity. Many (56.0%) of the livestock owners did not recall the history of the mass death of young animals. No-specific symptoms such as excess salivation were reported by 14.1% of respondents and loss of appetite, weakness, and diarrhea by 26.1% of participants together with signs of mass death of young animals and abortion. However, there was no significant difference between these variables and the serological documentation of WNV or RVFV infection. The statistical insignificance association of these signs and symptoms with outcomes of anti-RVFV and anti-WNV IgG, the signs and symptoms might arise due to other diseases such as brucellosis, Q-fever, toxoplasmosis, leptospirosis, and salmonellosis (FAO, 2021).

In the cattle seroprevalence study, WNV IgG-antibodies presence using ELISA was not confirmed by more advanced methods used to detect actual viral infection. Direct detection of the WNV from various animal species would give an improved understanding of the epidemiological situation in east Africa. Serologically measured anti-WNV IgG antibodies in cattle in other African countries also support the possibility that infection causes clinical disease in these hosts like in birds, horses, crocodiles, and humans where many clinical signs are shown. WNV occurrences in other species besides cattle elsewhere indicate the virus to be prevalent in birds, equine as well as in human populations (Glaser, 2004). The results of this study suggest the possible circulation of WNV warrants performing further studies in Ethiopia with other species.

Many east African countries depend on the pastoral livestock production system for milk and meat production. Therefore, livestock diseases, including RVFV may impact international livestock marketing and may harm the economics of a country like Ethiopia which depends mainly on the livestock besides the direct impact on human health (Pratt *et al.*,2004). In this study, an unknown disease characterized by high fever, joint and back pains transmitted from

sick animal to human after consumption of milk and meat mentioned in one of the sub-district the FGDs (Nip Nip) that bring forward occurrence of zoonotic disease such as RVF. Besides, responses of the FGDs indicated that mosquitoes such as *Aedes spp* bite both humans and animals during daytime in the forest areas, where similarly they bite humans at the forest, where that increases the transmission of vector-borne viruses such as RVF from animal to human. The other risk factor where humans could get the infection from animals directly would be the close contact between the humans and animals in the area. In the group discussion, participants indicated the possible importation of diseases from North and South Sudan together with their livestock.

6.2. **Community based seroprevalence of YFV, CHIKV, and ZIKV Infections in Human**

Reports about arboviral infections and outbreaks in humans in Ethiopia have been documented since the 1960s, yet the overall epidemiological situation in the country remains unknown (Ardoin *et al*., 1976; Sérié *et al*., 1968). In humans, this is the first epidemiological study conducted in Gambella Regional States of Ethiopia focusing on serological arboviral screening. Only a few studies on arboviruses have been conducted in SNNPR, Oromia, Diredawa, Somalia, and Amhara regions of Ethiopia and were mainly of part outbreak investigations *(*Ardoin *et al., 1976;* Endale *et al*., 2020; Lilay *et al*., 2017; Mengesha *et al*., 2018; Woyessa *et al*., 2013).

In the current community-based human seroprevalence study, a 2.9% prevalence of YFV IgG positivity was found among the tested serum samples. Apart from one subject, all three YFV IgG-positive individuals have no history of residency or travel outside the Gambella region. This indicates that YFV is endemic to the study area and likely circulates at low levels and might be reserved in the sylvatic cycle. Besides, three of the four individuals reported a history of working in the forest area for farming. According to the FGDs result, the vector ecology of *Aedes* mosquito and the majority of YFV seropositive individual working area indicate the possible sylvatic circulation. The current seroprevalence of IgG antibodies against YFV is in line with a previous study conducted in Djibouti where they found a prevalence of 1.5% of YFV IgG+ antibodies in blood samples (Andayi *et al*., 2014). However, another study from five locations of Ethiopia revealed that the seroprevalence of

YFV among people was 0.6% in pooled samples (Mengesha *et al*., 2018) which is lower than the present. The higher rate of daily commuters from neighboring countries (South Sudan, Sudan), migration of refugees, and seasonal movement of Fullani /Fellata pastoralists from north and West Africa could explain the higher seroprevalence that was found in this study. In contrast, our finding showed a lower anti-YFV IgG antibody response compared to other seroprevalence studies conducted in South Omo, Ethiopia (Endale *et al*., 2020) that resulted in 49.5% of individuals were seropositive for YFV IgG antibodies. The high prevalence of IgG in south Omo might be vaccine-related anti-body or directly related with the outbreak occurred in 2012/2013 in the area. High seroprevalence of YFV IgG antibodies was also found in Southern Ethiopia, the Borena area (12.5% of participants) (Nigussie *et al*., 2020) where this could be also sample was taken from febrile patients that elicit a high antibody and in Kenya (6% of participants) (Kwallah *et al*., 2015).

Almost all of the CHIKV IgG+ individuals' were engaged in farming activities in the forest area that might increase the probability of being bitten by infected mosquitoes were adapted to sylvatic areas. In the current study, a seroprevalence of 15.6% against CHIKV-specific IgG was detected among all participants which are in agreement with another study from India with a seroprevalence of 15% of CHIKV IgG antibodies (Kawle *et al*., 2017). On the other hand, the result of the current seroprevalence proportion of CHIKV IgG is lower compared to results from a rural community study of CHIKV in Brazil (18.3%) (Cunha *et al*., 2017) and 51% (Dias *et al*., 2018) from Latin America and 73.1% from East Sudan, Kassala area (Mohamed *et al*., 2019), a study from Ethiopia (43.6%) South Omon zone(Endale *et al*., 2020), and 23% of Metema area of Amhara region (Ferede *et al.*, 2021). In contrast, Khartoum state in Sudan and Southern Mozambique reported only a low seroprevalence of CHIKV IgG antibodies of 2.2% and 4.3%, respectively (Alfadil *et al*., 2017) (Gudo *et al*., 2015) yet, these seroprevalence studies were conducted in febrile patients. Hence, the current study differs where it screened only healthy subjects. The majority of the CHIKV IgG+ cases in this study were between 31-40 years of age, which is similar to findings from another CHIKV seroprevalence study in Tanzania (Kajeguka *et al*., 2016), this might show. The highest IgG seroprevalence of CHIKV was found in males which were fivefold (22.6%) higher compared to females. These findings were in agreement with another study that showed males are more susceptible than females (Azami *et al*., 2013; Sissoko *et al*., 2008).

On the other hand, other seroprevalence studies observed the opposite trend, where females were more often CHIKV IgG+ compared to males (Kawle *et al*., 2017; Mohanty *et al*., 2013). Anti-IgG antibody against CHIKV was significantly associated with the agro pastoralist lifestyle compared to a pastoralist one. The discrepancy with results from other studies might be due to sample size and proportions of the various categories such as gender and age. Furthermore, the participating community has various different habits, behaviors, occupations, traditions and local practices that would exposed them to the virus.

In the current study, there is serological evidence of human exposure to ZIKV in the two districts of the Gambella region. Overall, the prevalence of ZIKV IgG+ antibodies was found in 41 individuals (27.3%). Only six individuals had a history of traveling or residing outside of Gambella. Almost all individuals reported visiting forest areas for their farming activities. Significantly, the current ZIKV IgG+ prevalence was higher compared to study results conducted in Ethiopia in 2018 (0.4%) (Mengesha *et al*., 2018), Taken together, this study confirmed that ZIKV is endemic in this area and needs attention from public health agencies. A large fraction of the ZIKV IgG+ cases were found in the Lare district, which is 11 fold higher, compared to the ZIKV IgG+ cases in Itang special district. This could be due to the fact the district is the main entry point from South Sudan to Ethiopia where a temporary residential location and a registration place for refugees from South Sudan is located, movement of people across the borders is high. Neighboring countries such as Kenya have also reported high seroprevalence of ZIKV IgG+ antibodies(Labeaud *et al*., 2008; Sutherland *et al*., 2011).

Significantly higher ZIKV IgG+ antibodies were found in females in this study which is similar to a study conducted elsewhere (Victor *et al.*, 2018). Studies indicated that females in the sexually active age group are more likely to get ZIKV than males; sexual transmission is the most probable cause *(*Coelho *et al.*, 2016). Prevalence of ZIKV in gender-wise difference is also observed in mouse modeling *(*Duggal *et al*., 2018) as female mouses are more affected.

In general, a large fraction of the age groups 18-30 and 31-40 years was ZIKV IgG+ compared to individuals in the age group ≥41 years, which is in line with a study result from Zambia where study participants between 24-44 years of age showed a higher trend of ZIKV

IgG seropositivity compared to older participants (Babaniyi *et al.*, 2015). This result could be related to differences in mobility, occupation, and immune status between age groups. Participants with a history of residence or travel outside of Gambella also showed a higher trend for being ZIKV IgG+ which is in agreement with other study results (Babaniyi *et al*., 2015; Porse *et al.*, 2018). Participants who have a history of working in the forest areas were more likely to be ZIKV IgG+. Notably, pastoralists were found to be significantly more affected compared to agro-pastoralists which is in line with another study from Kenya (Chepkorir *et al*., 2019). This could be due to the tradition of the pastoralists and their lifestyle associated with long-distance movements with their livestock in search of pasture or water and exposes them more to forest areas and mosquito breeding sites close to water sources. Participants with informal education were more likely to have ZIKA IgG+ antibodies relative to those who have attended formal education. This might correlate with lower knowledge of disease transmission and the source of infection as shown by a study conducted in Nigeria (Ndibuagu, 2018).

6.3. Community awareness and experiences of health workers concerning mosquito-borne viral diseases

The results of this study revealed a lack of community awareness about different mosquitoes-borne viral diseases in general, although the study area can be considered as a high risk. However, the study participants have a high level of knowledge about malaria. There are many possible explanations for the lack of community awareness about mosquitoes-borne viral diseases. In areas like Gambella, where malaria is endemic, it should not be surprising to find awareness of people on mosquito-borne diseases to be focused mainly on malaria and other mosquitoes-borne diseases to remain unrecognized. Moreover, in resources limited countries, there is a high chance of misdiagnosis of mosquitoes-borne viral infections due to the lack of routine diagnostic tests and the non-specific nature of clinical symptoms of febrile-causing diseases (Ayorinde *et al*., 2016).

The previous study which focused on community knowledge, attitudes, and practices on one of the arbovirus diseases YF in Southern Ethiopia indicated that community members had great difficulty in differentiating between YF and malaria (especially confusion with

falciparum malaria)*(*Legesse *et al*., 2018). A study in Tanzania also revealed that many community members believed that most instances of fever are due to malaria and the community had a low level of awareness about other non-malaria febrile illnesses like RVF or DF despite the endemicity of these diseases(Chipwaza *et al*., 2014). Contrariwise, very few studies showed relatively good awareness of arbovirus diseases(Dhimal *et al*., 2014).

Almost all the participants in this study recognized *Anopheles* mosquito as the one that transmits malaria, which is locally known as *"*Nyise/Bewo". They also correctly recognized its breeding sites, biting time, and prevention using bed nets. However, few individuals argued that *Anopheles* bites both during the night and during the daytime in a dark place or inside the house. Conversely, the present study showed a better knowledge of the *Anopheles* vector compared to a study conducted in Jamaica(Alobuia *et al*., 2015).

The discussants also are familiar with the *Aedes* mosquito, as they have identified it as black-and-white color forest "Nyise", *which* breeds inside water bodies in a forest area during the rainy season. The participants from different kebeles also underlined that *Aedes* mosquito bites both humans and their animals during the daytime in forest areas or near water bodies, but it does not come to their home like that of *Anopheles* frequently.

A community-based study in rural Cambodia also revealed a high level of knowledge regarding *Aedes* breeding and biting time(Kumaran *et al*., 2018).

However, the majority of the participants expressed that they do not know what kind of disease the *Aedes* transmits following biting except resulting in intermittent irritating itching, which implies that the communities in the present study had no information regarding the role of *Aedes* mosquito in the transmission of any known disease. A previous study in Jamaica also showed very poor community knowledge of *Aedes* mosquito as the vector for DF to assess the vectors' knowledge (Alobuia *et al*., 2015). In a study conducted in Kongwa and Kilombero districts in Tanzania, a very small percent of participating community members were aware of the RVF vector (Shabani *et al*., 2015), suggesting the difficulty about prevention of RVF, which has a similar indication where mosquitoes would have transmitted diseases between animals and humans during the discussion. A study carried out to assess the awareness of DF vectors showed that people were not aware of the role of *Aedes* in the transmission of the disease and it also showed the existence of confusion with other

mosquitoes (Egedus *et al*., 2014). A study in the YF endemic area of Ethiopia also showed a low level of community knowledge about the vectors of YF and its mode of transmission(Legesse *et al*., 2018). The communities' knowledge of the *Anopheles* and *Aedes* mosquitoes biting in the area would play a big role in the tackling of malaria and other viral diseases.

Health workers from Etang health center expressed their concerns about the high risk of occurrence of YF in the area, because of the frequent migration of refugees/pastoralists from YF endemic border countries, primarily from South Sudan and Kenya to Gambella. The proximity of Gambella to countries like South Sudan, where repeated mosquito-borne viral disease outbreaks have been reported (Markoff, 2013), leads to the high risk of mosquito-borne viruses being introduced in the area. The environment is risky because the migration of refugees, animals, and wildlife across the border between South Sudan and the Gambella Region, as well as the ecological suitability of the region for mosquito vectors, would highly contribute to the occurrence and transmission of mosquito-borne viruses.

The community-based FGDs also indicated that there were participants who mentioned some of the clinical signs of mosquito-borne viral disease despite the fact the signs and symptoms are more or less similar to each other and counted as malaria cases. Study participants' responses from some of the Lare and Itang community-based FGDs suggested the occurrence and transmission of mosquitoes-borne viral diseases in the area.

Taken together, evidence collected in this study points towards the occurrence and transmission of mosquito-borne viral diseases in the area, which would not be surprising given the high risk for these diseases to occur. Unexpected outbreaks of major mosquitoes-borne viral diseases such as YF, DF, and RVF is not uncommon in East African countries since the early 1950s(Legesse *et al*., 2018; Lilay *et al*., 2017; Onyango *et al*., 2004; Sanders *et al*., 1998; Wamala *et al*., 2012; WHO, 2018a; Woyessa *et al*., 2013). However, in many cases, infection with mosquitoes-borne viruses causes subclinical or clinical signs/symptoms that are confused with other diseases such as malaria (Ayorinde *et al*., 2016; Gardner and Ryman, 2010) which was also reflected during the focus group discussion and warrants strengthening of surveillance.

Overall, in the present FGDs study, the diagnosis of febrile cases, in general, is rarely supported or confirmed by laboratory tests, reflecting the limitations of the health service delivery system and the shortage of laboratory facilities observed during the study period. This is likely leading to underdiagnoses and reporting of the actual presence of mosquito-borne viral diseases. Upon health workers' discussion, the poorly equipped laboratories in the region are not able to provide simple and urgently needed routine disease diagnosis; and even ruling out typhoid fever is a big challenge in the area. This kind of problem is also common in other African countries (Amarasinghe *et al.*, 2011; Ayorinde *et al*., 2016; Chipwaza *et al*., 2014).

This FGDs study would provide important information on community awareness and health workers' experiences about major mosquitoes-borne viruses in the study area. However, the study was conducted in purposively selected districts of the Gambella Region and the findings cannot be generalized to all the districts of the Region.

In this study, there was an observation where all the YFV IgG+ were found CHIKV positive while only one common positive between ZIKV and CHIKV during the screening of samples. This indicates there is a double infection of one person with CHIKV and YFV. Similarly, a report by Endale *et al*.(2020) showed that 60% of the tested individuals revealed positive for both viruses in South Omo Ethiopia. This indicates the possible circulation of these two viruses thus; the relative increases in the magnitude of CHIKV IgG antibody among YFV IgG antibody positive categories might be due to the circulation of both viruses in the study area. However, the findings warrant further studies to come up with a better understanding of the correlation between CHIKV and YFV IgG^{+} antibodies.

6.4. Limitations of the Studies

After reviewing the available literature published so far conducted, this is the first study and document dealing with arboviruses in the Gambella region and some are unique to Ethiopia. It is believed that this study would provide baseline data on the epidemiology of YFV, CHIKV, ZIKV, WNF, and RVF in the area. Despite the above fact, this study has the following limitations.

The community-based study lacks quantitative information on the level of knowledge, attitude, and practices.

One limitation of the study is that we did not use confirmatory tests such as Plaque Reduction Neutralization Test (PRNT), or PCR as proof of the viral agent due to budget limitations. Despite the fact it was proposed to collect 238 serum samples from humans, it cannot be achieved due to the lack of volunteer blood sample donors in the research protocol. In addition, it was unable to screen all the serum samples for YFV and CHIKV also due to budget limitations. Thus, the number of serum samples screened may be a limitation of the study. Finally, it was not able to conduct IgM ELISA screening to help distinguish recent exposure vs. more distant viral infection. Another setback of this study is that RVFV and WNV study was conducted only on cattle, excluding other species of livestock that would enable the comparison of the viruses' distributions in the available potential hosts.

7. CONCLUSION AND RECOMMENDATIONS

7.1. Conclusion

Detection of RVF and WNF antibody using indirect ELISA warrants further active case findings in cattle

The antibody-based ELISA study revealed the possible occurrence of RVF and WNF among the cattle population in the Gambella Region, which might show the circulation of the viruses in reservoir hosts in the area. Significantly, high IgG+ of both viruses was observed among those with a history of abortion. An increasing trend of IgG+ of both viruses was also observed among those that have a history of mass death of young animals and age 3.5 years and above. Comparatively, the high IgG+ to RVFV and WNV infections among the age group of 3.5+ years may show that these viruses have occurred in the study area recently. In this RVF and WNF study, a higher seroprevalence of IgG antibodies to RVF virus infection was observed compared to the WNF virus in cattle. Many of the cattle owners reported the occurrence of mass abortion in their herds, a finding significantly associated with RVFV seropositivity. In all the cattle serological examinations result there was no significant association between the seroprevalence and the cattle age, sex, or sampled locations.

YFV, CHIKV, and ZIKV serological examination revealed there have been possible circulation within the community, that initiate further active case investigations

The laboratory finding in this study showed the community-based seroprevalence of YFV, CHIKV, and ZIKV in two districts of the Gambella region, South West Ethiopia. Hence, the results revealed the circulation of these viruses within the community. Although the result of seroprevalence in different categories varies, the following can be concluded. CHIKV IgG+ antibodies were found to be significantly associated with the agro-pastoral community compared to the pastoral one. The seroprevalence of ZIKV IgG antibodies was found to be significantly higher in females and than in males which could be due to sexual transmission.

It is also found that ZIKV IgG+ cases were more prevalent in Lare compared to Itang special district. Therefore, it is an important step to include testing options in those health facilities, laboratories and to implement mosquito-borne viral disease prevention and control programs.

Community members and health workers do not have adequate information on mosquitoes-borne viral diseases in the area

The present study showed that community members and health workers in the study areas do not have adequate information on mosquitoes-borne viral diseases. On the other hand, evidence collected in this community-based and health workers FGDs study suggests the occurrence and transmission of mosquitoes-borne viral diseases in the present study area. Heath workers emphasized the lack of laboratory and diagnostic facility even malaria and other bacterial diseases are a major challenge in the area. In this FGDs study, all the discussants mentioned malaria, typhoid fever, unknown cause of diarrhea, and skin diseases as the major public health problems in the study area. The community-based FGDs result indicated major clinical signs of YF in particular and identified the presence of the primary vectors of many arboviruses in the area. Hence, discussants could able to confirm the presence of both *Anopheles* and *Aede* mosquitoes in the area. Discussants identified *Anopheles* as the vector of malaria while unable to mention the disease name transmitted through *Aedes* but, they mentioned it as it bites both humans and animals during the daytime in forest areas and causes skin itching to humans. Uniquely community participants from Pakag, a village bordering South Sudan, indicated that *Aedes* can also cause a malaria-like disease that can kill within a few days. Health workers from Etang health center described that in 2016, an outbreak of unknown disease causing fever and jaundice occurred and killed seven individuals in a village close to a South Sudan refugee camp.

Future Research Directions

Upon the current research Results and Output the possible future research directions include:

a. Detection of IgM from febrile humans and cattle
b. Studies on other livestock populations, wild animals, reservoir hosts, and vectors for better understanding the transmission dynamicity as well as the status of the viruses.

c. Active antigen detection of the viruses using molecular techniques, geospatial study on vector distribution, other domestic animals, and reservoir hosts
d. Refinement of other clinically similar diseases with arboviruses in both humans and animals
e. Research on quantitative-based surveillance on arboviruses for measuring the level of awareness (knowledge, attitude, and practice) to elucidate the quantitative level of information.

7.2. Recommendations

- There is a need to strengthen population-based surveillance of mosquito-borne viruses (YFV, CHIKV, RVFV, WNV, and ZIKV) and potential vectors, creating awareness among health care providers and community members about their agents, mode of transmissions, and taking preventive measures is indispensable.
- Seroprevalence study of RVFV and WNV on other species of domestic animals should be considered
- It is also important to appreciate the need for providing diagnostic testing for the health institutions of the region to ensure early detection and preparedness for these public health threats.
- Studies on the detection of active cases in domestic animals, humans, wild animals, and potential vectors using advanced techniques are highly acclaimed, for better future forecasts, and reductions of possible outbreaks
- The health system in the area should consider a one health approach in the prevention of arboviral infections in the national disease prevention and preparedness program.
- Looking for microcephaly cases in the area for ZIKV is recommended in new-borne individuals
- Location and occupation-based risk factors also specifically considered in future studies for ZIKV

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Annexes

Appendix 1: cattle information collecting sheet

s/n	Name of owner	code	woreda	kebele	village	Species	sex	age	HA	HMDY	hEXCE	parity	remark	remark

Sheet ----------

Appendix 2. Risk factor assessment on arboviruses exposure in Gambella Regional State, South Western Ethiopia

Risk factor assessment on arboviruses exposure in Gambella Regional State, South Western Ethiopia
Community-based serological test)
Name of Participant: _____code._____Woreda_____Kebele_____ Village_____ Date _____/_____/_____
Please kindly provide information for the following general questions

1	Sex: 1. Male 2. Female
2	Age: _____years
3	Ethnicity: 1. Nuer 2. Anywaa 3. Komo 4. Opow 4. Others (specify) _____
4	Religion: 1. Protestant 4. Orthodox 3. Muslim 4. Catholic 5. Others(specify) _____
5	Educational Status: 1. Illiterate 2. Read only 3. Read and write 4. Secondary and above 5. Other _____
6	Occupation: 1. Pastoralist 2. Agro pastoralist 4. Others (specify) _____
7	Duration of stay in this kebele: _____
8	History of residence in other countries Yes 2. No
9	If Q8 yes, where and when: Where _____When_____months/years
10	Do you have any travel history to other country/area (like South Sudan, Kenya, Uganda/other areas within Ethiopia): 1. Yes 2. No
11	If yes where and when: Where _____; When _____

12	History of working/traveling to areas like forest: 1. Yes 2. No
13	If Q12 yes, when _____
14	Do you know this mosquito (Aedes mosquitoes' picture demonstration): 1. Yes 2. No
15.	Where they (Aedes mosquito) are found/breed? _____
16	History of biting by this mosquito (*Aedes* mosquitoes' picture observation): 1. Yes 2. No
17	If Q16 yes, where and when: 1. Night at home; 2. Day time around home; 3. Day time in Forest area; 4. Other specify _____
18	If Q 16 yes, do you think biting by this mosquito causes a disease: 1. Yes 2. No 3. I don't know
19	If yes to question 18, what type of disease?_____
20	Any history of chronic diseases: 1. Yes 2. No
21	If Q 20 yes, mention the disease: _____
22	Are there primates (monkey and apes around your residence or grazing areas)? 1. Yes 2. No
23	History of vaccination for yellow fever (check list/card): 1. Yes 2. No

Date collected by_____ date_____signature_____

Appendix 3. Basic focal group discussants information

የማህበረሰብ የቡድን ተወያዮች መሰረታዊ መረጃ/Community focal group discussants basic information

ተ/ቁ/sn	ስም/name	እድሜ/age	ጾታ/sex	ስራ/occupation	ቀበሌ/sub-district	የጋብቻ ሁኔታ/marital status	ጎሳ/ethnic base

የጤና ባለሙያዎች መሰረታዊ መረጃ /Health workers basic information

ተ/ቁ/sn	ስም/name	እድሜ/age	ጾታ/sex	ሙያ/ qualification	ቀበሌ/sub-district	የስራ ልምድ/work experience

Appendix 4. Focal group Discussion check lists

a) Main public health problems in this area
b) Ordering of listed diseases
c) Major clinical signs and symptoms of listed diseases
d) Diseases transmitted through mosquito, other than mentioned above
    a. Additional diseases transmitted through mosquitoes if available
e) Do you know these mosquitoes (*Anopheles* and *Adese*) (picture based discussion)
    a. Have you seen these in this area
    b. What kind of diseases they transmit
    c. Where they live
    d. When they bit
    e. Do you know what kind of disease the zebra like mosquito transmit
f) Which of these eyes is abnormal
    a. The top or the bottom
g) What kind of prevention method is recommended

The discussion was started by giving equal chance for the participants, and it was conducted by assisted

Appendix 5. Illustrations for focal group discussion

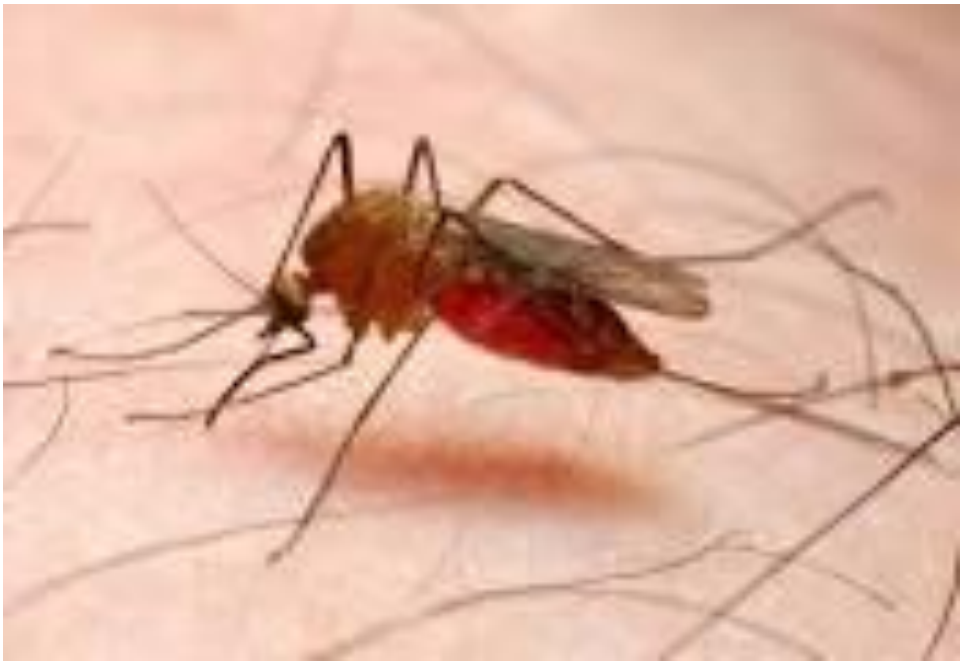
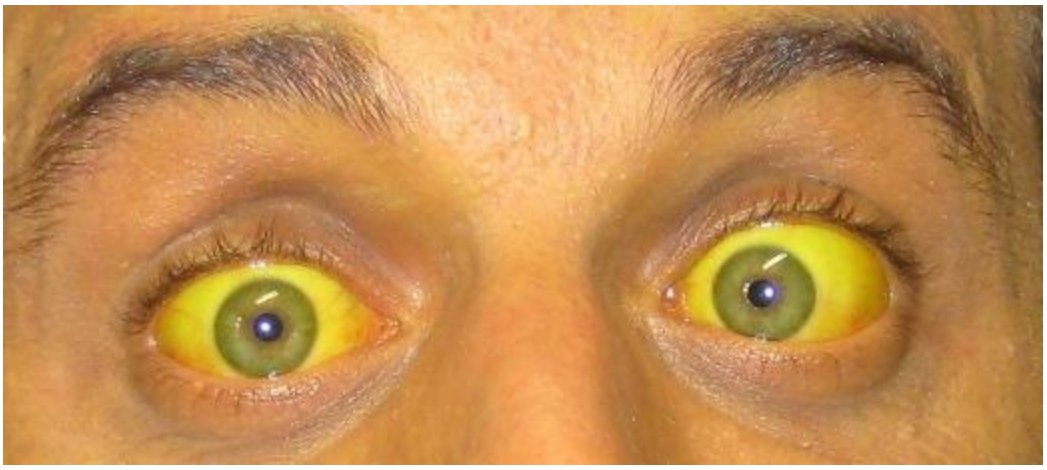


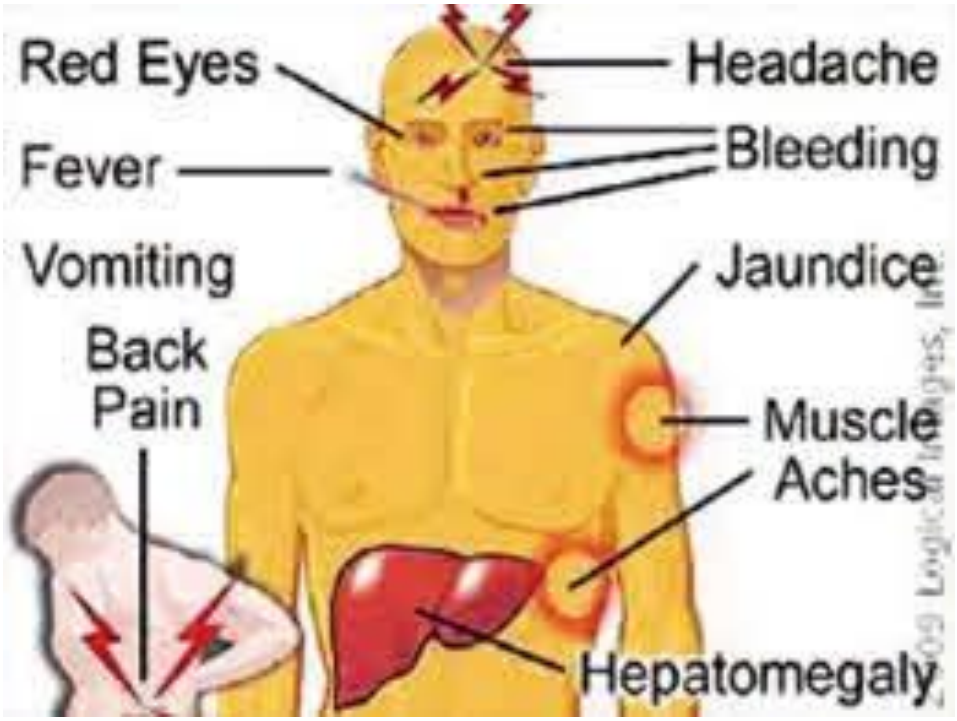
Figure 18. Figures used during the Group discussion in the study areas.

a. Picture of *Anopheles* mosquito (Anopheles mosquito stock image, 2014)

b. Picture of *Aedes* mosquito (Shutterstock, 2017)



C, Picture of Yellowish eye after yellow fever virus (GTR, 2019)



d, Arboviral signs in an affected person (The Star, 2018)

Appendix 6. Cow Rift Valley fever Virus IgG ELISA Kit

Range: Qualitative

Sensitivity: Qualitative

Application: For qualitative detection of RVFV in cow serum, plasma, Tissue Homogenaates and cell culture supernatants.

Principe of the Assay

A 96 well plate has been pre-coated with the target antigen. Controls or test samples are added to the appropriate wells and incubated. Free components are washed away with wash buffer. HHRP conjugated detection reagents are used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colour product that changes to yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the RVFV-IgG amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and the presence of RVFV-IgG can be determined.

Materials used (for 96 well)

Kit components

1. one pre-coated 96-well microplate ((8*12 well strips)),
2. Positive control 0.5ml,
3. Negative control: 0.5ml
4. Wash buffer (30X): 20ml. dilution: 1:30
5. Sample diluent buffer: 6ml
6. HRP conjugate reagent (RTU) : 6ml
7. Stop solution: 6ml
8. TMB substrate A: 6ml
9. TMB substate B: 6ml
10. Plate sealer: 2
11. Hermetic bag: 1

Materials used

1. 37^0C incubater
2. Microplate reader (Wavelength 450 nm)
3. Multi and Single channel pipette and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. 1.5ml tubes
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

A. Preparation of samples and reagents

Isolates the test samples soon after collecting and analyze immediately at 1/5 dilution (within 2 hours) or aliquot and store at -20^0C or -80^0C for long-term storage.

1. Sample

The serum samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4^0C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000Xg for 20 minutes. Analyze the serum immediately or aliquot and store at -20^0C or -80^0C. (The storage of samples should be undiluted. Once ready to analyze, thaw samples and dilute 1/5).

2. Wash buffer

Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e add 20 ml of concentrated wash buffer into 380ml of distilled water).

B. Assay procedure

Equilibrate the kit components and samples to room temperature prior to use.

1. Set positive/negative controls, test sample and control (zero/blank) wells on the pre-coated plate and record their positions.
2. Aliquot 50µl of the negative and positive controls into the set wells. Leave one well as the control (zero) blank well.

3. Aliquot 50µl of appropriately diluted samples into the test sample wells. Samples should be diluted 1/5. Add the solution at the bottom without touching the sidewalls of the well. Shake the plate gently to mix the contents.
4. Seal the plate with a cover and incubate at 37^0C for 30 minutes
5. Remove the cover and discard the solution. Wash the plate 5 times with 1X wash buffer. Fill each well completely with Wash buffer (300µl) using a multi-channel Pipette or auto washer (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
6. Add 100µl of HRP conjugate reagent into each well (except the blank well). Add the solution at the bottom of each well without touching the sidewall.
7. Seal the plate with a cover and incubate at 37^0C for 60 minutes.
8. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 5.
9. Aliquot 50µl of TMB substrate A into each well, and then add 50µl of TMB Substrate B. Vortex the plate gently on an ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37^0C for 15 minutes. Avoid exposure to light.
10. Add 50µl of Stop solution into each well to stop the enzyme. It is important that the Stop solution be mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.

11. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

C. Analysis

1. **Calculations**:

   Mean absorbance of the positive control should be ≥1.00

   Mean absorbance of the negative control should be ≤0.10

   CUT OFF value=Negative control + 0.15

2. **Interpretation of results**:

   If the positive control value is ≥ 1.00 and the negative control value is ≤ 0.10, the test is valid; otherwise, the test is invalid.

   If O.D. of samples < CUT OFF, the test samples are considered negative.

   If O.D. of samples ≥ CUT OFF, the test samples are considered positive.

D. Precautions

1. Before using the kit, centrifuge the **tubes** to bring down the contents trapped in the lid
2. **Avoid foaming**, bubbles when mixing, or **reconstituting** components.
3. If crystals have formed in the concentrated **Wash Buffer**, warm to room temperature and mix gently until the crystals have completely **dissolved.**
4. It is recommended measuring each controls and samples in duplicate.
5. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells do not let the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
6. Ensure plates are properly sealed or covered during incubation steps.

7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. Do not use expired components from different kit.
10. The TMB Substrate B is easily contaminated: protect from light and work under sterile conditions when handling the TMB substrate solution. Equilibrate at room temperature prior to use. Unreacted substrate should be colorless or very yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

Appendix 7. Cattle West Nile Virus IgG ELISA Kit

Range: Qualitative

Sensitivity: Qualitative

Application: For qualitative detection of WNV-IgG in cow serum, plasma, Tissue Homogenates and cell culture supernatants.

Principe of the Assay

A 96 well plate has been pre-coated with the target antigen. Controls or test samples are added to the appropriate wells and incubated. Free components are washed away with wash buffer. HHRP conjugated detection reagents are used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colour product that changes to yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the WNV-IgG amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and the presence of WNV-IgG can be determined.

Materials used (for 96 well)

Kit components

1. one pre-coated 96-well microplate ((8*12 well strips)),
2. Positive control 0.5ml,
3. Negative control: 0.5ml
4. Wash buffer (30X): 20ml. dilution: 1:30
5. Sample diluent buffer: 6ml
6. HRP conjugate reagent (RTU) : 6ml
7. Stop solution: 6ml
8. TMB substrate A: 6ml
9. TMB substate B: 6ml
10. Plate sealer: 2
11. Hermetic bag: 1

Materials used

1. 37^0C incubater
2. Microplate reader (Wavelength 450 nm)
3. Multi and Single channel pipette and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. 1.5ml tubes
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

A. Preparation of samples and reagents

Isolates the test samples soon after collecting and analyze immediately at 1/5 dilution (within 2 hours) or aliquot and store at -20^0C or -80^0C for long-term storage.

1. Sample

The serum samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4^0C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000Xg for 20 minutes. Analyze the serum immediately or aliquot and store at -20^0C or -80^0C. (The storage of samples should be undiluted. Once ready to analyze, thaw samples and dilute 1/5).

2. Wash buffer

Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e add 20 ml of concentrated wash buffer into 380ml of distilled water).

B. Assay procedure

Equilibrate the kit components and samples to room temperature prior to use.

1. Set positive/negative controls, test sample and control (zero/blank) wells on the pre-coated plate and record their positions.
2. Aliquot 50µl of the negative and positive controls into the set wells. Leave one well as the control (zero) blank well.

3. Aliquot 50µl of appropriately diluted samples into the test sample wells. Samples should be diluted 1/5. Add the solution at the bottom without touching the sidewalls of the well. Shake the plate gently to mix the contents.
4. Seal the plate with a cover and incubate at 37^0C for 30 minutes
5. Remove the cover and discard the solution. Wash the plate 5 times with 1X wash buffer. Fill each well completely with Wash buffer (300µl) using a multi-channel Pipette or auto washer (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
6. Add 100µl of HRP conjugate reagent into each well (except the blank well). Add the solution at the bottom of each well without touching the sidewall.
7. Seal the plate with a cover and incubate at 37^0C for 60 minutes.
8. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 5.
9. Aliquot 50µl of TMB substrate A into each well, and then add 50µl of TMB Substrate B. Vortex the plate gently on an ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37^0C for 15 minutes. Avoid exposure to light.
10. Add 50µl of Stop solution into each well to stop the enzyme. It is important that the Stop solution be mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.

11. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

C. Analysis

3. **Calculations**:

   Mean absorbance of the positive control should be ≥1.00

   Mean absorbance of the negative control should be ≤0.10

   CUT OFF value=Negative control + 0.15

4. **Interpretation of results**:

   If the positive control value is ≥ 1.00 and the negative control value is ≤ 0.10, the test is valid, otherwise, the test is invalid.

   If O.D. of samples < CUT OFF, the test samples are considered negative.

   If O.D. of samples ≥ CUT OFF, the test samples are considered positive.

D. Precautions

1. Before using the kit, centrifuge the **tubes** to bring down the contents trapped in the lid
2. **Avoid foaming** or **bubbles** when **mixing** or **reconstituting** components.
3. If crystals have formed in the concentrated **Wash Buffer**, warm to room temperature and mix gently until the crystals have completely **dissolved.**
4. It is recommended measuring each controls and samples in duplicate.
5. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells do not let the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
6. Ensure plates are properly sealed or covered during incubation steps.

7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. Do not use expired components from different kit.
10. The TMB Substrate B is easily contaminated: protect from light and work under sterile conditions when handling the TMB substrate solution. Equilibrate at room temperature prior to use. Unreacted substrate should be colorless or very yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

Appendix 8. Human Yellow Fever Virus IgG (YFV-IgG) ELISA kit

Range: Qualitative

Sensitivity: Qualitative

Application: For qualitative detection of YFV-IgG in human serum, plasma, Tissue Homogenaates and cell culture supernatants.

Principe of the Assay

A 96 well plate has been pre-coated with the target antigen. Controls or test samples are added to the appropriate wells and incubated. Free components are washed away with wash buffer. HRP conjugated detection reagents are used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colour product that changes to yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the YFV-IgG amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and the presence of YFV-IgG can be determined.

Materials used (for 96 well)

Kit components

1. one pre-coated 96-well microplate ((8*12 well strips)),
2. Positive control 0.5ml,
3. Negative control: 0.5ml
4. Wash buffer (30X): 20ml. dilution: 1:30
5. Sample diluent buffer: 6ml
6. HRP conjugate reagent (RTU) : 6ml
7. Stop solution: 6ml
8. TMB substrate A: 6ml
9. TMB substate B: 6ml
10. Plate sealer: 2
11. Hermetic bag: 1

Materials used

1. 37^0C incubater
2. Microplate reader (Wavelength 450 nm)
3. Multi and Single channel pipette and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. 1.5ml tubes
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

A. Preparation of samples and reagents

Isolates the test samples soon after collecting and analyze immediately at 1/5 dilution (within 2 hours) or aliquot and store at -20^0C or -80^0C for long-term storage.

1. Sample

The serum samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4^0C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000Xg for 20 minutes. Analyze the serum immediately or aliquot and store at -20^0C or -80^0C. (The storage of samples should be undiluted. Once ready to analyze, thaw samples and dilute 1/5).

2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e add 20 ml of concentrated wash buffer into 580ml of distilled water).

B. Assay procedure

Equilibrate the kit components and samples to room temperature prior to use.

1. Set positive/negative controls, test sample and control(zero/blank) wells on the pre-coated plate and record their positions.
2. Aliquot 50µl of the negative and positive controls into the set wells. Leave one well as the control (zero) blank well.
3. Aliquot 50µl of appropriately diluted samples into the test sample wells. Samples should be diluted 1/5. Add the solution at the bottom without touching the sidewalls of the well. Shake the plate gently to mix the contents.
4. Seal the plate with a cover and incubate at 37^0C for 30 minutes
5. Remove the cover and discard the solution. Wash the plate 5 times with 1X wash buffer. Fill each well completely with Wash buffer (300µl) using a multi-channel Pipette or auto washer (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
6. Add 50µl of HRP conjugate reagent into each well (except the blank well). Add the solution at the bottom of each well without touching the sidewall.
7. Seal the plate with a cover and incubate at 37^0C for 30 minutes.

8. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 5.
9. Aliquot 50µl of TMB substrate A into each well, and then add 50µl of TMB Substrate B. Vortex the plate gently on an ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37^0C for 15 minutes. Avoid exposure to light.
10. Add 50µl of Stop solution into each well to stop the enzyme. It is important that the Stop solution be mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
11. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

C. Analysis

5. **Calculations**:

   Mean absorbance of the positive control should be ≥1.00

   Mean absorbance of the negative control should be ≤0.10

   CUT OFF value=Negative control + 0.15

6. **Interpretation of results**:

   If the positive control value is ≥ 1.00 and the negative control value is ≤ 0.10, the test is valid, otherwise, the test is invalid.

   If O.D. of samples < CUT OFF, the test samples are considered negative.

   If O.D. of samples ≥ CUT OFF, the test samples are considered positive.

D. Precautions

1. Before using the kit, centrifuge the **tubes** to bring down the contents trapped in the lid
2. **Avoid foaming** or **bubbles** when **mixing** or **reconstituting** components.
3. If crystals have formed in the concentrated **Wash Buffer**, warm to room temperature and mix gently until the crystals have completely **dissolved.**
4. It is recommended measuring each controls and samples in duplicate.
5. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells do not let the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. Do not use expired components from different kit.
10. The TMB Substrate B is easily contaminated: protect from light and work under sterile conditions when handling the TMB substrate solution. Equilibrate at room temperature prior to use. Unreacted substrate should be colorless or very yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

Appendix 9. Human Chikungunya Virus IgG (CHIKV-IgG) ELISA kit

Range: Qualitative

Sensitivity: Qualitative

Application: For qualitative detection of CHIKV-IgG in human serum, plasma, Tissue Homogenaates and cell culture supernatants.

Principe of the Assay

A 96 well plate has been pre-coated with the target antigen. Controls or test samples are added to the appropriate wells and incubated. Free components are washed away with wash buffer. HRP conjugated detection reagents are used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colour product that changes to yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the CHIKV-IgG amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and the presence of CHIKV-IgG can be determined.

Materials used (for 96 well)

Kit components

1. one pre-coated 96-well microplate ((8*12 well strips)),
2. Positive control 0.5ml,
3. Negative control: 0.5ml
4. Wash buffer (30X): 20ml. dilution: 1:30
5. Sample diluent buffer: 6ml
6. HRP conjugate reagent (RTU) : 6ml
7. Stop solution: 6ml
8. TMB substrate A: 6ml
9. TMB substate B: 6ml
10. Plate sealer: 2
11. Hermetic bag: 1

Materials used

1. 37^0C incubator
2. Microplate reader (Wavelength 450 nm)
3. Multi and Single channel pipette and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker

6. 1.5ml tubes
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

A. Preparation of samples and reagents

Isolates the test samples soon after collecting and analyzes immediately at 1/5 dilution (within 2 hours) or aliquot and store at -20^{0}C or -80^{0}C for long-term storage.

3. Sample

The serum samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4^{0}C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000Xg for 20 minutes. Analyze the serum immediately or aliquot and store at -20^{0}C or -80^{0}C. (The storage of samples should be undiluted. Once ready to analyze, thaw samples and dilute 1/5).

4. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e add 20 ml of concentrated wash buffer into 580ml of distilled water).

B. Assay procedure

Equilibrate the kit components and samples to room temperature prior to use.

1. Set positive/negative controls, test sample and control(zero/blank) wells on the pre-coated plate and record their positions.
2. Aliquot 50µl of the negative and positive controls into the set wells. Leave one well as the control (zero) blank well.
3. Aliquot 50µl of appropriately diluted samples into the test sample wells. Samples should be diluted 1/5. Add the solution at the bottom without touching the sidewalls of the well. Shake the plate gently to mix the contents.
4. Seal the plate with a cover and incubate at 37^{0}C for 30 minutes

5. Remove the cover and discard the solution. Wash the plate 5 times with 1X wash buffer. Fill each well completely with Wash buffer (300µl) using a multi-channel Pipette or auto washer (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
6. Add 50µl of HRP conjugate reagent into each well (except the blank well). Add the solution at the bottom of each well without touching the sidewall.
7. Seal the plate with a cover and incubate at 37^0C for 30 minutes.
8. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 5.
9. Aliquot 50µl of TMB substrate A into each well, and then add 50µl of TMB Substrate B. Vortex the plate gently on an ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37^0C for 15 minutes. Avoid exposure to light.
10. Add 50µl of Stop solution into each well to stop the enzyme. It is important that the Stop solution be mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
11. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

C. Analysis

7. **Calculations**:

Mean absorbance of the positive control should be ≥1.00

Mean absorbance of the negative control should be ≤0.10

CUT OFF value=Negative control + 0.15

8. **Interpretation of results**:

   If the positive control value is ≥ 1.00 and the negative control value is ≤ 0.10, the test is valid, otherwise, the test is invalid.

   If O.D. of samples < CUT OFF, the test samples are considered negative.

   If O.D. of samples ≥ CUT OFF, the test samples are considered positive.

D. Precautions

1. Before using the kit, centrifuge the **tubes** to bring down the contents trapped in the lid
2. **Avoid foaming** or **bubbles** when **mixing** or **reconstituting** components.
3. If crystals have formed in the concentrated **Wash Buffer**, warm to room temperature and mix gently until the crystals have completely **dissolved.**
4. It is recommended measuring each controls and samples in duplicate.
5. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells do not let the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. Do not use expired components from different kit.
10. The TMB Substrate B is easily contaminated: protect from light and work under sterile conditions when handling the TMB substrate solution. Equilibrate at room

temperature prior to use. Unreacted substrate should be colorless or very yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

Appendix 10. BLOCKING OF BINDING (BOB) ASSAY

ELISA-based assay to detect specific Zika antibodies (Abs) in sera or plasma of Zika virus (ZIKV)-immune individuals and to differentiate from prior exposure to DENV. The assay is based on the principal that the presence of antibodies in the serum of individuals that react with the non-structural-1 (NS1) protein of ZIKV inhibit the assay signal, which is based on recognition of ZIKV NS1 by the monoclonal antibody (ZKA35). Of note, ZKA35 specifically binds ZIKV NS1 but not dengue virus (DENV) NS1.

REAGENTS

- ZKA35-HRP labeled (Absolute antibody #Ab01036-10.0-BOB)
- Cold (non-conjugated) antibody: ZKA35-rIgG1 (unlabeled; Absolute antibody #Ab01036-10.0-CTL)
- Antigen (ZIKV NS1 e.g. Meridian #R01636 or Native Antigen Company # ZIKV-NS1-100)
- Phosphate Buffered Saline (PBS)
- ubstrate: SureBlue Reserve TMB 1-Component Micro well Peroxi (e.g. Bioconcept #5120-0083)
- Stop solution: 1% HCl
- Nunc-Immuno plates - 96-well plate, MaxiSorp (e.g. Sigma #M9410-1CS) (=Nunc 439454)

(Alternative plate e.g. Product #9018: Corning 96 Well Clear Flat Bottom Polystyrene High Bind Microplate, 25 per Bag, without Lid, Nonsterile)
Note: Do not use 384 well plate for this assay, as the background would be too high.

Blocking buffer/Diluent	1% BSA *(e.g. Sigma, #* A9430*)* in PBS
Bicarbonate buffer	1.59g Na2CO3 (e.g. Sigma-Aldrich #71345) 2.93g NaHCO3 (e.g. Sigma, #71627) 0.*2g NaN3 (e.g. Sigma, #71290)* 1 L milliQ-H2O pH 9.6
Washing buffer:	*0.5% PBS* *0.05% Tween 20*

(I) DETERMINATION OF ZKA35-HRP WORKING DILUTION (EC70)

To determine EC70 of ZKA35-HRP concentration to be used in the competition assay for binding to coated ZIKV NS1 in your experimental condition:

- Coat plates with antigen (1 µg/ml ZIKV NS1) in PBS, 50 µl/well
- Incubate over night at 4°C, plate must be sealed
- Wash plates 2x with Washing buffer (250 µl/well)
- Add blocking buffer, 200 µl/well
- Incubate 1 h at room temperature
- Wash plates 2x with Washing buffer (250 µl/well)

Add **50 µl sample/well** of: (1) sera/plasma to test (1:10 diluted in diluent), duplicates recommended
2) 3 wells **negative control**: control plasma/sera diluted 1:10 in assay diluent or diluent only
3) 3 wells **positive control:** 5 µg/ml ZKA35 mAb in control plasma/sera that was diluted 1:10 in assay diluent)

- Row 1 and 2: diluent
- Row 3 and 4: Negative control serum diluted 1:10 in diluent
- Incubate 1 h at room temperature
- Do not wash
- Add ZKA35-HRP: starting dilution 1:10 , 1:3 serial dilution of 12 points in diluent, using **50 µl ZKA35-HRP/well** (***)
- Incubate 15 min at room temperature
- Wash plates 4x with Washing buffer (250 µl/well)
- Dispense substrate solution SureBlue Reserve TMB, 40 µl/well
- Incubate at room temperature (time depending on signal: until reaching a visible bright blue color, e.g. 10-15 min. After adding the stop solution (below), a final OD value of 2-3 should be reached in the upper plateau of the dilution series.)
- Dispense stop solution 1% HCl, 40 µl/well
- Read absorbance at 450 nm
- Determine the concentration of ZKA35-HRP to be used in the BOB assay corresponding to 70% of the maximal OD level by interpolating a curve fitted with a 4-parameter nonlinear regression. Consider the ZKA35-HRP input dilution (***), see example data, below.

Example of plate layout

A												
B		sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	sample 7	sample 8	sample 9	negative control	
C		sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	sample 7	sample 8	sample 9	negative control	
D		sample 10	sample 11	sample 12	sample 13	sample 14	sample 15	sample 16	sample 17	sample 18	negative control	
E		sample 10	sample 11	sample 12	sample 13	sample 14	sample 15	sample 16	sample 17	sample 18	positive control	
F		sample 19	sample 20	sample 21	sample 22	sample 23	sample 24	sample 25	sample 26	sample 27	positive control	
G		sample 19	sample 20	sample 21	sample 22	sample 23	sample 24	sample 25	sample 26	sample 27	positive control	
H												
	1	2	3	4	5	6	7	8	9	10	11	12

- Incubate 1 h at room temperature
- DO NOT WASH
- Add ZKA35-HRP at the concentration of the amount corresponding to 70% of the maximal OD level (EC70 established above (***)), 50 µl/well (in ALL wells, including negative and positive controls)
- Incubate each plate 15 min at room temperature
- Wash plates 4x with Washing buffer (250 µl/well)
- Add substrate solution SureBlue Reserve TMB, 40 µl/well
- Incubate at room temperature
- Dispense stop solution 1% HCl, 40 µl/well
- Read absorbance at 450 nm

Appendix 11. Amharic version of focal group discussion consent form

በጋምቤላ ሕዝቦች ብሔራዊ ክልላዊ መንግስት በ ኢታንግ/ ላሬ/ ወረዳ በትንኝና በዝንብ አማካኝነት የሚተላለፉ፤ በሰውና በእንስሳት ላይ ጉዳት በሚያደርሱት ላይ የሚደረግ (የሕብረተሰብ ውይይት)፤

የወረዳ ስም…….

የቀበሌ ስም…….

የመንደር ስም…….

መለያ ቁጥር……

ሙሉ ስሜ -..... ይባላል፡፡

የመጣሁት ከ----------ሲሆን በ አዲስ አበባ ዩኒቨርሲቲ የድህረ ምረቃ ት/ቤት ተማሪ ነኝ፡፡

ርዕስ ፡- በኢታንግ/ ላሬ ወረዳ በትንኝና በዝንብ አማካኝነት የሚተላለፉ፤ በሰውና በእንስሳት ላይ ጉዳት በሚያደርሱት በሽታዎች ስርጭት፤ ቁጥጥሩ እና ሕክምና ዙሪያ ስላዉ እውቀት ፤ አመለካከት እና ትግበራ የሚደረግ ጥናት

የጥናቱ አላማ ፡- በኢታንግ/ ላሬ/ ወረዳ በትንኝ እና በዝንብ አማካኝነት የሚተላለፉ፤ በሰውና በእንስሳት ላይ ጉዳት በሚያደርሱ በሽታዎችን ስርጭት፤ ቁጥጥሩ እና እውቀት ፤ አመለካከት እና ትግበራ ስላለዉ መረጃ መገምገምን ይመለከታል፡፡

የጥናቱ ዓላማ ከዚህ በላይ የተጠቀሰው የተጠቀሰ ሲሆን እርስዎ የጥናቱ ተሳታፊ እንዲሆኑ ተመርጠዋል፡፡መረጃውን የምንሰበስበው ከርስዎ እና ከሌሎች በእጣ በተመረጡ ሰዎች ጋር በጋራ የሚደረግ ውይይት ሲሆን ለዚህ ውይይት ከአንድ ሰዓት እስከ አንድ ሰዓት ተኩል የሚወስድ ይሆናል፡፡ የመወያያ ነጥቦቹ የሚያተኩሩት 1)በአካባቢያዊ፣ 2)ስለ ትንኝ ወለድ ቫይረስ በሽታወች ምንነት እና አጠቃላይ እውቀት፤ በሽታ መከላከል እና ሕክምና 3) ስለ ቫይረስ በሽታወች ያለቸውን አጠቃላይ አመለካከት፤ 4) ስለ ቫይረስ በሽታወች አጠቃላይ መከላከል እና የመከላከል አጠቃላይ ትግበራወች እእ 4) እንዲሁም ተዛማች ነገሮችን በተመለከተ ይሆናል፡፡ በዚህ ጥናት ላይ ግለሰቦች የሚሰጡት አስተያየት ሚስጥራዊነት የሚጠበቀ ሲሆን በምንም መልኩ በግል አይወጣም፡፡ ሁሉም መረጃወች በቡድን ያካባቢዉን ሃሳብ ብቻ የሚያንጸባረቁ ሲሆነ ያንድም ሰው ስም በጥናቱ ውጤት ላይ አይመዘገብም፡፡ ሁላችሁም የምትሰጡት አስተያየት በነጻነት እና የምታውቁትን ሲሆን የሚሰጡን መልሶች ለጥናታችን ውጤታማነት ወሳኝ እንደሆን ባክብሮት እንገልጻለን፡፡ በዚህ አጋጣሚ በዚዚ ጥናት ላይ ለመሳተፍም ሆነ ላለመሳተፍ ሙሉ መብቶ የተጠበቀ ነው፡፡ በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ በሚቀጥለው ስፍራ ላይ በመፈረም ያረጋግጡልን፡፡

የፈቃደኝነት ስምምነት

ከላይ የተሰጠውን መረጀ አንብቤያለሁ /ተነቦልኛል፡፡ የጥናቱ አላማ ምን ምን እንደሆነ አውቄያለሁ"በመሆኑም በጥናቱ ውስጥ ተሳታፊ በመሆኔ የሚደርስብኝ ችግር እንደሌለ አረጋግጫለሁ፡፡ ስለዚህም በጥናቱ ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ ::

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ስም ፊርማ ቀን

ሰለአረጉልን ማንኛው ትብብር እናመሰግናለን ::

Appendix 12. Blood sample consent form/English version

Addis Ababa University School of Graduate

BLOOD SAMPLE CONSENT

DESCRIPTION: My name is Getahun Asebe, Graduate student at Addis Ababa University. Dear participant you are kindly invited to participate in a research study entitled "A sero-epidemiological study of Arboviruses in Gambella Regional State, South Western Ethiopia**".** The purpose of this study is to investigate the serological epidemiology of arboviruses, as we believe that some risk factors are available and predispose humans for virus infections. Here we want to look the virus presence and distribution across this woreda in some selected kebeles/villages.

Dear participant, you will be asked to *provide a sample of blood (3 ml). The blood will be* taken with a needle from your arm for your standard clinic visit.

After we complete our tests, we would like to save any leftover blood for future related research on arthropod borne. We will keep your blood sample under deep freeze and will be stored with a number assigned to it instead of your name. The number will be linked to your name, which means you can withdraw from this study at any time.

The results of the study of your samples will be used for research purposes only and you will not be told the results of the tests.

I consent to my samples being saved for future research [ ]

I do not consent to my samples being saved for future research [ ]

RISKS AND BENEFITS: The risks associated with this study are slight discomfort or bruising from the blood draw and the possible loss of confidentiality if your data or information is inadvertently disclosed outside of this study. You will not benefit from the study, as this is not a treatment study. We cannot and do not guarantee or promise that you will receive any benefits from this study, because the intention of the study is not for commercial use.

TIME INVOLVEMENT: Your participation in this experiment will take approximately 15 minutes for the blood draw (which will be done as part of your annual visit).

PAYMENTS: *You will not be paid to participate in this study*.

PARTICIPANT IS RIGHTS: If you have read this form and have decided to participate in this project, please understand your participation is voluntary and you have the right to withdraw your consent or discontinue participation at any time without penalty or loss of benefits to which you are otherwise entitled.

The results of this research study may be presented at scientific or professional meetings or published in scientific journals. However, your identity will not be disclosed**.**

If applicable: You have the right to refuse to answer particular questions.

If you continue to participate in this study, please put you name and signature

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Name Signature Date

Appendix 13. Consent and information sheet for blood collection from cattle

ለጥናቱ ተሳታፊዎች የሚሰጥ መግለጫ

የጥናቱ አርዕስት፡ የተለያዩ በሽታዎች በትንኝ ንክሻ አማካኝነት የሚተላለፉ መንስኤያቸው በቫይረስ መጠነ-ስርጭትና ተያያዥ አጋላጭ መንስኤዎች በጋምቤላ ሕዝቦች ብሄራዊ ክልላዊ መንግስት በላሬና ኢታንግ ልዩ ወርዳ

የጥናቱ ተመራማሪዎች

*ጌታሁን አሰበ (የ’PhD’ ተማሪ፣ ሞባይል፡ 251-911-571579፣ ኢ-ሜይል፡ getahunasebe@gmaill.com

ዶ/ር መንግስቱ ለገሰ (የጥናቱ ዋና አስተባባሪ፣ ሞባይል፡ 0911345908)

*ዶ/ር ገዛኸኝ ማሞ (በእንስሳት ሕክምና ኮልጅ ዋና አማካሪ፤ ሞባይል፡0911678195)

**ዶ/ር ወልደአረጋይ እርኩ (ሞባይል፡ 0911453297)

ዶ/ር ግርማይ መድህን (ሞባይል፡ 0911834982)

አድራሻ

አክሊሉ ለማ ፓቶባዮሎጂ መካነ ጥናት/*እንስሳት ሕክምና ኮልጅ/**ጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ፣ አዲስ አበባ

ስልክ፡ 251 -11-276-30-91 (ቢሮ)

የፖስታ መልዕክት ሳጥን ቁጥር፡ 1176 አዲስ አበባ፣ ኢትዮጵያ

ለምርምሩ ሥራ የገንዘብ ድጋፍ የሚያደርገዉ ድርጅት፡ አዲስ አበባ ዩኒቨርሲቲ፣ የጥናትና ምርምር ቢሮ

መግቢያ (በትንኝ ንክሻ ስለሚተላለፉ በሽታዎች ምንነት)

የተለያዩ በሽታዎች በትንኝ ንክሻ አማካኝነት የሚተላለፉ መንስኤያቸው በቫይረስ የሆኑ በሽታ ነዉ አሉ፡፡ ከነዚህ በሽታዎች መካከል የስምጥ ሸለቆ የትኩሳት በሽታ (ሪፍት ቫሊይ ፌቨር ይገኝበታል)፡፡ ይህ በሽታ ሰውን ጨምሮ የተለያዩ እንስሳዎችን ማለትም (ከብት፣ ጎሽ፣ በግ፣ ፍየል እና ግመልን) ያጠቃል፡፡ቫይረሱ ወደ ሰውነት ከገባ ብኋላ ከ2-5 ቀን ውስጥ ምልክት ማሳየት ይጀምራል፡፡ በዚህ የተያዘ ሰው ትኩሳት፣ የሰውነት መንቀሳቀስ ማነስ፣ እራስ ምታት፣ ከባድ የሆነ የጡንቻና የመገጣጠሚያ ሕመም፣ ብርሃን የመፍራት እና የምግብ ፍላጎት መቀነስ ምልክት ያሳያል፡፡ ተውከት፣ ማቅለሽለሽ፤ ነስር፣ የቆዳ መቅላት ምልክቶችም አልፈው አልፈው ይታያሉ፡፡ ይህ በሽታ የጠና ካልሆነ በ2 ሳምንት ውስጥ መሻሻል ያሳያል፡፡በሽታው የጠና ከሆነ ደግሞ የደም ተውከት እንዲሁም ያለማቋረጥ የደም መፍሰስ ሊስከትም ይችላል፡፡ የከፉ ደረጃ ላይ የደረሱ በሽተኞች ባብዛናዉ ይሞታሉ ሆኖም ጥቂቶች ከረጅም የመታመም ሂደት ብኋላ ሊተርፉ ይችላሉ፡፡ የዚህ በሽታ ለላዉ ውስብስብነቱ ከእይታ ጋር እስከ አይነስዉርነት ድረስ ከባድ ጠባሳ ጥሎ ሊልፍ ይችላል፡፡ ግራ የመጋባት እና የአይምሮ ችግርም ሊያስከትል ይችላል፡፡

<u>በሽተዉ በእንስሳት ላይ የሚያሳየዉ ምልክት</u>

በርግጥ ምልክቶቹ ከዝርያ ዝርያ እና ከርግዝና ሁኔታ ይያያዛሉ፡፡ እንደወረርሽኝ ሲከሰት ከፍተኛ የሆነ ውርጃ ያስከቲላል፣ በእድሜ ትንንሽ የሆኑት ላይ ሞትን ያመጣል፡፡ እርጉዝ በሆኑ በግና ከብት ላይ እስከ መቶ በመቶ የሚድረስ ውርጃ ያስከትላል፡፡ ግልገሎች እና ጥጃዎች ላይ ትኩሳት በማምጣት እና በማዳከም ወዲያው ይገላል፡፡ በትላልቆች ላይ እንደየቅደም ተከተላቸው ከ20-10 በመቶ የሚደርስ በግና ከብት ላይ ሞትን ያመጣል፡፡ በትላልቅ በግና ከብት ላይ በተጨማሪም ብዛት ያለው ምራቅ፣ የምግብ ፍላጎት መቀነስ፣ ድካም፣ ተቅማጥ፣ የአፍንጫ ፈሳሽን የመሳሰሉት ምልክቶች ያታያሉ፡፡

አገራችን ኢትዮጵያ ለበሽታዉ ተጋለጭ ከሆኑ አገሮች ዉሰጥ አንዷ ስትሆን ይህ በሽታ በአጎራባች ሃገራትና ማለትም በደቡብ ሱዳን፤ ኬንያ እና ሶማሊያ ውስጥ ብዙ ጊዜ ይከሰታል፡፡ በርግጥ በእኛ ሀገር እስካሁን ከቦረና አካባቢ የተወሰነ ምልክትና እና አንድ ሁለት ሪፖርት ተደርጓል፡፡ ይሁን እነጂ የበሽታዉ መጠነ-ስርጭትና ተያያዥ አጋላጭ መንስኤዎች በተለያዩ የሀገራችን አካባቢዎች ያልተጠና እዲሁም በጋምቤላ ሕዝቦች ክልል እስካሁን ያልተጠናና የማይታወቅ በመሆኑ ለዚሁ መልስ ይሆን ዘንድ በተለያዩ ለደቡብ ሱዳን ቅርብ በሆኑት የጋምቤላ ክልል ወረዳዎች ውስጥ በተለያዩ የቤት እስሳት ላይ የደም ናሙና በመዉሰድ ጥናት ማድረግ አስፈላጊ ሆኖ ተገኝቷል፡፡

የጥናቱ አላማ

የዚህ ጥናት አላማ መሰረት ያደረገው በዋናነት የስምጥ ሸለቆ ትኩሳት(ሪፍት ቫሊ ፌቨር) እና ተዛማጅ በትንኝ ንክሻ አማካኝነት የሚተላለፉ ቫይረሶች መጠነ-ስርጭትና ተያያዥ አጋላጭ መንስኤዎች በኢታንግ ልዩ ወረዳና በላሬ ወረዳ ውስጥ በተመረጡ ቀበሌዎች ላይ ባሉ በተለያዩ የቤት እንስሳት ላይ ጥናትና ምርምር ማድረግ ነው፡፡

የጥናቱ ተሳታፊዎች

በዚህ ጥናት ዉስጥ የሚሳተፉ የተለያዩ የቤት እንስሳት (ከብት) ላይ በላሬና እና በኢታንግ ልዩ ወረዳዎች በተመረጡ ቀበሌዎች ውስጥ ነው፡፡

የጥናቱ ተሳታፊዎች የሚሰጡት መረጃና ሂደት

በዚህ ጥናት ላይ መሳተፍ ሙሉ ፈቃደኝነት ላይ የተመሠረተ ነው፡፡ ስለሆነም በመጀመሪያ በጥናቱ እንዲሣተፉ ፈቃደኝነትዎን በትህትና እንጠይቃለን፡፡በዚህ ጥናት ለመሳተፍ ከፈቀዱ ለአስር ደቂቃ ያህል ለጥያቄዎች ምላሽ ይሰጡናል፡፡ ከዚያም 5 ሚሊ ሊትር (አንድ የሻይ ማንኪያ) የደም ናሙና ከቤት እንስሳዎ/ዎቹ ላይ እንወስዳለን፡፡

በጥናቱ በመሳተፍ የሚመጣ ጉዳት

የጥናቱ ተሳታፊ እንስሳዎች ላይ ችግር ይከሰታል ተብሎ አይጠበቅም፡፡ ሆኖም ግን ጥቂት የደም ናሙና በምንወስድበት ጊዜ ትንሽ የህመም ስሜት ሊሰማቸው ይችላል፡፡

በጥናቱ በመሳተፍ የሚገኝ ጥቅም

በጥናቱ በመሳተፍ ምክንያት የሚገኝ ገንዘብ የለም ሆኖም ግን በጥናቱ ለሚሳተፉ የቤት እንስሳዎ የዉስጥና የውጭ ጥገኛ የተለያዩ የመድሃኒ ቶችን በነጻ እንሰጣለን እናክማለን፡፡ በተላየየ የባክቴሪያል ኢንፌክሽን ለተጠቁት እንስሳትም አስፈላጊዉን የሕክምና እርዳት እናደርጋለን፡፡ በተጨማሪም ይህ ጥናት ለወደፊቱ የበሽታ ክትትልና ቁጥጥር ከፍተኛ እገዛ ስለሚያደርግ ከፍተኛ አስተዋጽኦ ያደርጋል፡፡

ምስጢርን ስለመጠበቅ

በጥናቱ ውስጥ የተሰበሰቡ ማናቸውም ግላዊ መረጃዎች ሚስጥራዊነታቸው የተጠበቀ ይሆናል፡፡ ከማንነትዎ ጋር በቀጥታ ተያያዥነት ያላቸው መረጃዎች ለምሳሌ ስምና ሌላ ነገሮች በጥናቱ ዉጤት ላይ አይታተምም፡፡

ከጥናቱ ስለመውጣትና ስለማቋረጥ

ይህ ጥናት በፈቃደኝነት ላይ የተመሰረተ እንደመሆኑ መጠን በማናቸውም ወቅት በፈቃደዎ ከጥናቱ መውጣት ይችላሉ፡፡ ከጥናቱ ቢወጡም እንኳ በማንኛዉም የቀደመ መብትዎ የተጠበቀ ነዉ፡፡

ጥያቄ ካለዎት

ከጥናቱ ጋር በተያያዘ ማናቸውም ጥያቄ ቢኖርዎ ከላይ በተጠቀሰዉ አድራሻ መሰረት ለጥናቱ ተመራማሪዎች ጥያቄዎን ማቅረብ ይቻላል፡፡

የጥናቱ ተሳታፊዎች የስምምነት ማረጋገጫ ቅጽ

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እኔ ስሜ ከላይ የተጠቀሰዉ በትንኝ ንክሻ የሚተላለፉ ቫይረሶች መጠነ-ስርጭትና ተያያዥ ኢጋላጭ መንስኤዎች ላይ በላሬ/በኢታንግ ልዩ ወረዳ ስለሚደረገዉ ጥናት በቂ መረጃ የተሰጠኝ ሲሆን የጥናቱ ተሳታፊ ለመሆን ከተስማማሁ ቃለመጠይቅ እንደሚጠየቅ እንድሁም 5 ሚሊ ሊትር የደም ናሙና ከቤት እንስሳየ ላይ እንዲወሰድ የተስማማሁ መሆኔን እገልጻለሁ፡፡

በጥናቱም በመሳተፌ ምክንያት ሊደርስብኝ የሚችል ችግር እንደሌለም ተገልጾልኛል፡፡ የደም ናሙናዉ ከስምጥ ሸለቆ ትኩሳት (ሪፍት ቫሊ ፌቨር) ና ሌሎች በትንኝ ንክሻ አማካኝነት ከሚተላለፉ ቫይረሶች ባሻገር የተረፈዉ የደም ናሙና ለከብቶች የውርጃ በሽታም ጥናትና ምርምር ጭምር እንደምዉል ተገልጾልኛል፡፡ በተጨማሪም እኔ የምሰጠዉ መረጃ በሚስጢር እንደሚያዝና ለስምጥ ሸለቆ ትኩሳት (ሪፍት ቫሊ ፌቨር) እና ሌሎች በትንኝ ንክሻ አማካኝነት የሚተላለፉ ቫይረሶች መጠነ-ስርጭትና ኢጋላጭ መንስኤዎች እና ለከእንስሳት ውርጃ በሽታ ጥናትና ምርምር ብቻ እንደሚዉል ተገልጾልኛል፡፡ ከእኔ ጋር ቀጥተኛ ግንኙነት ያላቸዉ ነገሮች እነደ ስም የመሳሰሉት በጥናቱ ዉጤት ላይ እንደማይታተም ተገልጾልኛል፡፡በጥናቱ መሳተፍ ሙሉ ለሙሉ በፈቃደኝነት ላይ የተመሰረተ መሆኑ ተገልጾልኛል፡፡ ስለዚህ በጥናቱ ለመሳተፍ መስማማቴን በፊርማዬ አረጋግጣለሁ፡፡

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Veterinary Medicine: Research and Reports

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ORIGINAL RESEARCH

Seroprevalence of Rift Valley Fever and West Nile Fever in Cattle in Gambella Region, South West Ethiopia

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Introduction: Rift Valley fever (RVF) and West Nile fever (WNF) are re-emerging mosquito-borne zoonotic diseases that cause public health and economic crises. Ethiopia shares borders with South Sudan and Kenya, where these diseases are often documented. The free movement of animals and humans across these borders expects to increase the spread of these diseases. The current study was conducted to assess the occurrence of these diseases in the Gambella region of Ethiopia.

Methodology: We collected a total of 368 cattle serum samples from the Lare district on the border of South Sudan and measured the presence of IgG antibody against RVF and WNF virus infections using enzyme-linked immunosorbent assays (ELISA).

Results: The prevalence of anti-RVF virus IgG antibody was 7.6% (95% CI: 5.3–10.82%), while that of anti-WNF virus IgG antibody was 5.4% (95% CI: 3.52–8.29%). In this study higher seroprevalence of IgG antibodies to RVF virus infection was observed comparing to the WNF virus in cattle. There was no significant association between the prevalence and the cattle age, sex or sampled locations.

Conclusion: The detection of IgG antibody to RVF and WNF virus infections in the Gambella region warrants further study of active case findings and the dynamics of transmission.

Keywords: serology RVF; WNF; cattle; Gambella, Ethiopia

Background

Arboviruses are among the 72% of emerging infectious viruses that originate from wildlife.[1] They transmit to different vertebrate hosts by arthropod species such as mosquitoes, ticks, sand flies and midges.[2] Climatic changes influence the transmission of the viruses associated with the life cycle of the transmitting vectors.[3]

Rift Valley fever virus (RVFV) and West Nile virus (WNV) are among the re-emerging viruses transmitted through the bite of infected *Aedes* and *Culex* mosquitoes respectively.[4,5] RVFV belongs to the family *Bunyavirida*, genus *Phlebovirus*.[6] RVFV was first identified in 1931 near the Great Rift Valley of Kenya.[6] Since then, this virus has caused many documented outbreaks in humans and livestock in many sub-Saharan African countries, in the Arabian Peninsula, and some Indian Ocean Islands.[7,8] RVFV infects a range of vertebrate hosts including humans, livestock, and wildlife, with ruminants being among the most affected hosts.[9] Infection with RVFV can result in severe disease that can lead to high morbidity and mortality in animals including abortions and stillbirths.[10,11]

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In humans, the virus causes neonatal mortalities and moderate influenza-like illness. Similarly, severe complications and small proportion of deaths may occur among some groups of infected people.[11–14]

Unusually, heavy and sustained rainfall leads to outbreaks of the disease by favoring breeding of the mosquito vectors.[15] Study reports documented presence of RVFV in some East African countries: South Sudan,[16] Kenya[10,17] Somalia, Tanzania and Sudan.[18] where some of them are neighbors of Ethiopia.

WNV is a single-stranded RNA virus that belongs to the family of *Flaviviridae*, genus *Flavivirus*,[19] and was first isolated from a febrile patient in the West Nile district of Northern Uganda in 1937. The virus causes disease mainly in birds, equines and humans.[20,21] Birds are important vertebrate amplifying hosts and their migratory pattern have greatly influenced the re-emergence and global spread of WNV. The virus infects the mosquitoes when they suck blood-meal from infected birds. Thereafter, the mosquitoes spread the virus on the next susceptible host they bite.[22] Humans and other mammals, especially horses, may develop clinical illness but usually are incidental or dead-end hosts because they do not produce significant viremia, and thus do not contribute to the transmission cycle.[23]

WNV also infects domestic animals such as cows.[24] WNV infections typically cause a mild febrile illness in humans. The main risk factors for WNV transmission includes environmental and anthropological factors that increase mosquitoes' population during heavy rain and flooding, irrigation, global warming and formation of ecologic niches that favor mass breeding of mosquitoes.[25]

WNV has been documented in 83 countries around the world, including several countries in Africa (e.g. Uganda, Kenya, Democratic Republic of Congo and Sudan[26,27]), the Middle East, Asia, and Australia.[28–30] In Ethiopia very few studies have been conducted regarding arboviruses infections in humans,[31–34] in spite of ample evidence regarding the importance of animals and human movement between neighboring countries in the spread of arboviruses.

The transmission dynamics between the hosts and reservoir, behavior of intermediate insect vectors population, animals and humans' movement and urbanizations are among the key factors that alter the epidemiology of many emerging and re-merging viral diseases. Similarly, the challenge of many arboviruses in the absence of adequate diagnostic facility and capacity in livestock is supposed to be significantly increased, especially in remote areas. Hence, due to many known and unknown factors, the arboviruses remain under-recognized and underreported in many developing worlds such as Africa including Ethiopia. Some of the factors so far mentioned as low awareness by health-care providers, the presence of other prevalent febrile illnesses, lack of diagnostic testing and regular systematic surveillance, and many other factors. The main hypothesis of selecting the present study area were: a) because of its proximity to South Sudan in which these viruses are already confirmed; b) the hot and humid climate favoring arboviral transmission, and conducive for vector breeding and c) the lack of RVF vaccine administration: Importantly, to date, the RVFV vaccine has not been previously administered to cattle in Ethiopia. Henceforth, the aim of this study was to determine the seoprevalence of RVF and WNF to assess possible RVFV and WNV circulation in Gambella Region, Southwest Ethiopia.

Materials and Methods

Study Area and Study Design

We conducted a cross-sectional study in the Gambella Region of Ethiopia between December 2018 and January 2019. Gambella is one of the nine regions of Ethiopia. The geographical location lies between latitudes 6° 22′ and 8° 30′ N, and longitudes 33° 10′ and 35° 50′ E. The elevation of Gambella region ranges from ~1000 to ~2000 m above sea level (masl) in the east, from ~500–900 masl in the center, and ~300–500 masl in the west.[35] Recent human population of the region is estimated to be 478,000.[36] The livestock census conducted in the region in the year 2018 indicated 285,102 cattle, 35,285 sheep, 107,083 goats, and 301,531 poultry.[37]

In the present study area, the Abigar cattle breed are the most dominant both in the Nuer and Anywaa zones of the region.[38] Among the indigenous genotypes known in the country, these cattle are known as "Sanga" located normally in the border area between Ethiopia and Sudan with larger extension in Ethiopia covering the Akobo area of Gambella.[39] Geographically this breed is located around the White Nile of Sudan and Ethiopia. The breed is mainly distributed in the neighboring lowlands of Southwest of Ethiopia. This breed is mainly reared and kept by the Nuer ethnic groups.[40,41] This breed of cattle is characterized by the unique features that are used in identifying the breed from

other indigenous breeds. The breed has large body size, long-curved horns, gray and white dominant coat colors as well as ease of management by all gender groups due to its recognized docile character. In addition, the breed has better production and reproduction capabilities despite the high heat load, recurrent drought and repeated disease prevalence in the region.[38] The African cattle breeds in general are known as more resistant to diseases including RVFV compare to European breeds.[42]

Lare is one of the 13 districts of the Gambella Region belonging to the Nuer Zone that borders South Sudan (Figure 1). The district was selected based on (a) its high cattle population, and (b) its adjacent to South Sudan where different arboviruses cases are previously reported including RVFV.[43] The district hosts pastoralists migrating from South and North Sudan (commonly called "Fallata" or "Fulani") in search of pasture and water for their livestock.[44] The indigenous pastoralists also travel far from their villages to the adjacent territories of South Sudan in search of pasture. Pastoralists have a tradition of presenting and accepting cattle to or from the South Sudan community during marriage ceremonies which have the potential to introduce diseases in the study area.

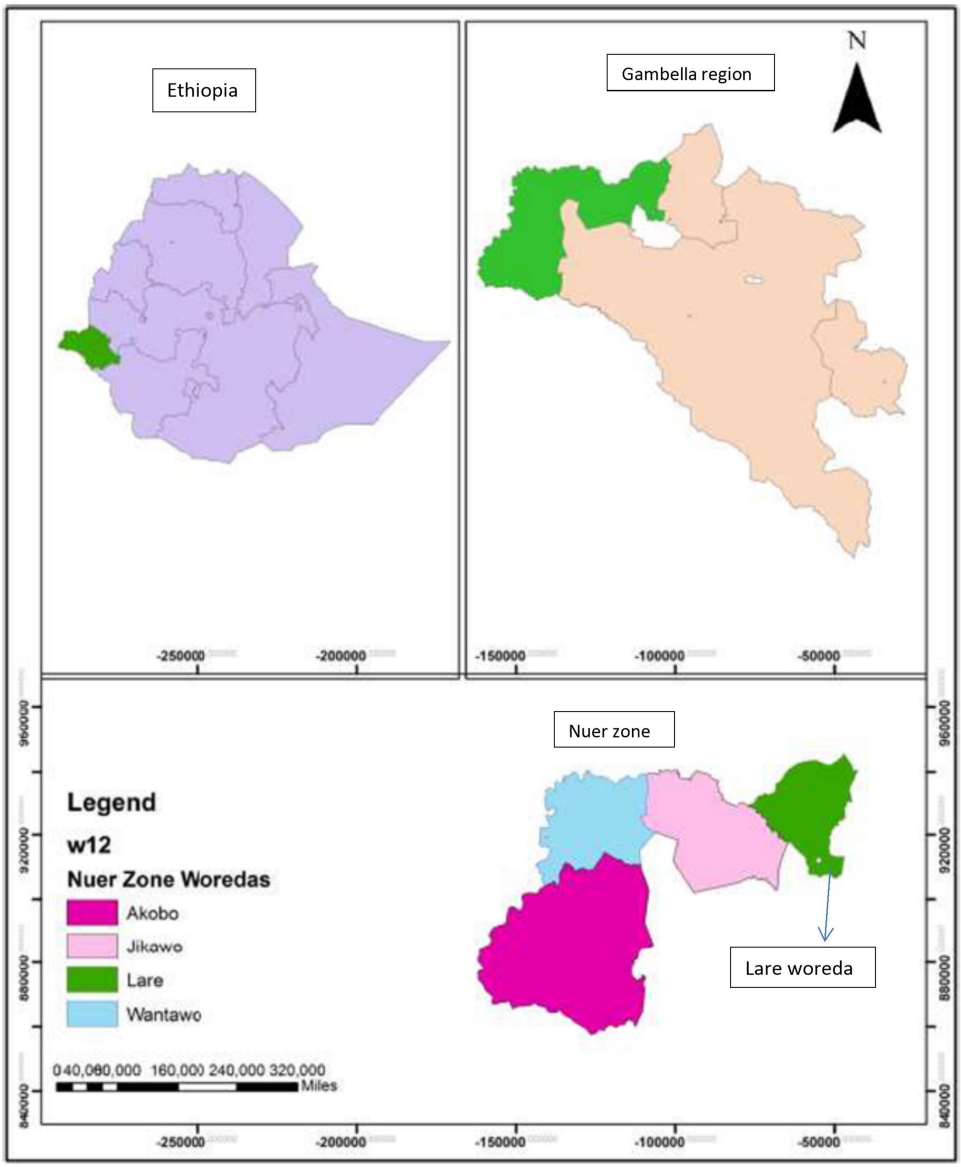

Figure 1 Map of study area (top left Ethiopia; Top right Gambella Region; bottom right Nuer zone and, Lare woreda with green color) "Woreda" is the local name representing the district.

Sample Size Estimation and Data Collection

The sample size was determined using the following formula: $N = \frac{Z^2 p(1-p)}{d^2}$, where Z = 1.96 corresponding value to the desired 95% confidence level, *p* is an estimate of the expected prevalence of infection, and *d* is the margin of error in the estimate.[45] In designing this study we took the estimate of *p* from Southwestern Uganda with a RVFV prevalence of 27%.[13] We added 20% to account for non-response of cattle owners about cattle during sampling, questioning and dropping of aggressive animals. These assumptions resulted in a minimum sample size of 368 cattle. Four pastoral sub districts or "kebeles" (the lowest government administrative structure in Ethiopia) were selected for the study. These kebeles were Pal Bol, Bilimkun, Kechi and Tandor. Each selection was based on the cattle population, proximity to South Sudan and their frequent contact with the pastoralists from North and South Sudan. Inclusion criteria for recruitment of study were cattle of both sexes and a minimum age of one year.

Based upon the age of puberty in local cattle breeds in the area, the study animals were classified into two groups: 1–3.5 years of age and 3.5+ years.[38] After obtaining the owners' consent, 5 mL blood sample was collected from selected cattle in the herd via the jugular venipuncture using a sterile vacutainer tube. Information such as age, sex, parity, herd size, history of mass abortion, history of mass death of young animals and some selected clinical signs regarding each animal (identified by a temporary paper-based identification code) was recorded separately using a checklist prepared as a mini questionnaire at the time of blood collection. Serum was separated and stored at −20°C until screening for IgG antibody to RVFV and WNV infections by ELISA.

Laboratory Analysis

The indirect ELISA-based type detection of IgG antibody against RVFV and WNV infections was conducted as per the manufacturer's protocol ready for each individual (Abbexa Ltd, Cambridge UK).[46] The diagnostic sensitivity and specificity have not been determined for these specific kits where they are only ready for research purposes. All the tests were conducted in duplicates. The results were read at an optical density (OD) of 450 nm using a 96-well ELISA plate reader (Multiskan™ FC Microplate Photometer), and interpreted as positive or negative based on the manufacturer's recommended cut-off values. Test validity was verified according to the manufacturer's manual when the mean OD of the positive control was ≥1 and when the mean OD of the negative control was ≤0.15. The cut-off value for each plate was calculated independently calculated where the cut-off value = negative control +0.15. Hence, any sample value greater than or equal to this value labelled as positive and any value less than the cut-off value scored as negative.

Statistical Analysis

All collected data cleared and entered into EpiData Software v.3.1 and analyzed using STATA Version 13.0. In the study herds, age, sex, and parity were used as explanatory parameters, while the history of mass abortion and the history of mass death of young animals together with other signs such as excess salivation, and loss of appetite and diarrhea were used to investigate clinical signs mainly about the RVF. In this study, the outcome variables were ELISA IgG antibody positive and negative.

The seroprevalence/apparent prevalence of IgG antibody elicited towards both RVFV and WNV were estimated by dividing the number of cattle with positive test results by the total number of tested cattle. Here the cattle included in the study were apparently healthy and sample was not taken from diseased animals where true prevalence could be determined. Univariable logistic regression was used to assess the crude association between the seropositivity of IgG antibody and the hypothesized individual potential risk factors such as age, sex, parity, and site, calculated with descriptive and analytical analysis using chi-square (χ^2) test. Multivariable logistic regression analysis was used to assess the effect of each of the independent variables on the outcome variable (seropositivity) after adjusting each independent variable for all other variables. A p-value below 0.05 was considered indicative of a statistically significant association.

Ethical Approval and Consent to Participate

The livestock owners were informed and aware about the purpose of the study, and that verbal informed consent was approved by the Institutional Review Board of the College of Veterinary Medicine and Agriculture, Addis Ababa University with the certificate reference no: VM/ERC/07/05/10/2018. Besides, permission to visit the study sites and to collect the blood sample from cattle was obtained from the Gambella Regional Livestock and Fishery Office,

district administration Office and community leaders of each study site. Blood sample collection was carried out under aseptic conditions by experienced veterinary laboratory technicians.

Results

Characteristics of the Study Cattle and Owners' Responses on Selected Clinical Signs

A total of 275 (74.7%) female and 93 (25.3%) male cattle were included in the current study. The majority were in the age groups of 1–3.5 years, the minimum and maximum age of the sampled cattle population was 1 and 20 years, respectively, and the average age was 3.9 years with a standard deviation of 3.23. Among 121 cows the overall mean of the birth rate was 3.0 calves per cow (standard deviation = 2.2). The number of births per cow ranged from 1 to 14 calves.

The animals came from 53 herds with a minimum and a maximum herd size of 7 and 67 animals, respectively, and a mean herd size of 23.2. Just over half (50.9%) of the herd owners described mass abortion in the area and 43% mentioned the deaths of young animals within their herd within the past three to five years. Among the herd owners, 43.4% mentioned the historical presence of mass death in their animals (Table 1).

Table 1 Characteristics of the Study Cattle and Owners' Responses About Selected Clinical Signs

Characteristics	Response Category	Number	Percent
Sex	Male Female	93 275	25.3 74.7
Age group (N=368)	1–3.5 years +3.5 years	222 146	60.3 39.7
Parity (N=275)	Yes No	121 154	44.0 56.0
Study kebele/site (N=368)	Pal Bol Kech Bilimkun Tandor	41 152 89 86	11.1 41.3 24.2 23.4
History of abortion (N=53 herds)	Yes No	27 26	50.9 49.1
History of mass death in your animals (N=53 herds)	Yes No	23 30	43.4 56.6
Previous signs and symptoms of excess salivation in sampled animals (N=368)	Yes No	52 316	14.1 85.9
Previous history of loss of appetite, diarrhea and excess nausea in sampled animals (N=368 sampled animals)	Yes No	96 272	26.1 73.9

Seroprevalence of RVFV Infection

Out of 368 analyzed serum samples, 28 (7.6%) (95% CI: 5.29–10.81%) were positive for IgG antibody to RVFV infection, which was significantly associated with a history of abortion (14.8%; p=0.041) compared to those cows had no history of abortion. However, there was no significant association between IgG positivity to the virus and the study kebeles (villages) (Table 2).

Seroprevalence of WNV Infection

Table 3 presents the seroprevalence of WNV infection stratified by different background characteristics. The seroprevalence of WNV-specific IgG antibody was detected in serum samples collected from 20 cattle (5.4%) (95% CI: 3.52–8.28%). No significant associations were observed between the findings of WNV antibody IgG and past recorded clinical signs (Table 3). However, significantly higher proportions of anti-WNV IgG antibody were observed in serum samples collected from cattle with a history of abortion as compared to those without a history of abortion (11.1% Vs 3.8%, p= 0.04)

Independent Predictors of Seropositivity for RVFV Infection

In a multivariate logistic regression analysis where sex, age, parity, sub districts, and selected clinical signs considered were taken as independent predictors, no significant association between the RVFV specific IgG antibody and these predictor variables were observed (Table 4).

Independent Predictors of Seropositivity for WNV Infection

Similar to the finding observed in modeling the odds of getting RVFV positive IgG, all the variables included in the multivariate logistic regression that investigated factors associated with the odds of getting WNV positive IgG did not result in statistically significant association with any one of the investigated risk factors (Table 5).

Table 2 Seropositivity for RVFV Infection in Cattle

Characteristics	Response Group	Total Tested	Sero Status of IgG for RVFV		P-value
			Positive No. (%)	Negative No. (%)	
Sex	Male Female	93 275	9 (9.7) 19 (6.9)	84(90.3) 256 (92.1)	0.384
Age group (N=368)	1–3.5 years 3.5+ years	222 146	16(7.2) 12(8.2)	206(92.8) 134 (91.8)	0.720
Parity (N=275)	Yes No	121 154	10 (8.3) 9(5.8)	111(91.7) 145(94.2)	0.432
Kebele	Pal Bol Kech Bilimkun Tandor	41 152 89 86	3 (7.3) 8(5.3) 5(5.6) 12 (13.9)	38(92.7) 144(94.7) 84(94.4) 74(86.1)	0.085
History of abortion (N=53 herds)	Yes No	27 26	4(14.8) 0 (0.0)	23(85.2) 26(100.0)	0.041
History of mass death in your animals (N=53 herds)	Yes No	23 30	3 (13.1) 1(3.3)	20(86.9) 29(96.7)	0.185
Previous signs and symptoms of excess salivation in sampled animals (N=368)	Yes No	52 316	6 (11.5) 22(7.0)	46(88.5) 294(93.0)	0.249
Previous history of loss of appetite, weakness, diarrhea and excess nausea in sampled animals (N=368 sampled animals)	Yes No	96 272	6 (6.3) 22 (8.1)	90(93.7) 250(91.9)	0.560
All		368	28(7.6)	340(92.39)	

Discussion

A limited number of studies on arboviruses in human[31–34] and livestock[47] have been carried out in Ethiopia. This seroprevalence study focused on RVFV and WNV infections in cattle in the Gambella region of southwestern Ethiopia. This region and the study districts were selected due to their proximity to South Sudan, which serves as the entry point for many refugees to Ethiopia as well as the main route for livestock movement between the adjacent territories.

The present study revealed 28 (7.6%) (95% CI: 5.29–10.81%) animals were seropositive for RVFV infection, while 20 (5.4%) (95% CI: 3.52–8.28%) were seropositive for WNV infection. Antibody to both viruses was found in cattle from all the four sub-district "kebeles". Comparing the anti-IgG of WNV and RVFV in this study, it has been found high prevalence of RVFV than WNV.

The seroprevalence of RVFV infection in cattle previously reported in another African country is 6.2% in the eastern region of the Democratic Republic of the Congo[48] which is similar to the result of current study. On the other hand, the seroprevalence observed in our study is lower than a report from the eastern parts of Ethiopia, Somalia region which is 17.9%[47] and from other African countries like Cameroon (9.4%),[49] Rwanda (16.8%);[10] Madagascar (25.8%)[50] and Mozambique (36.6%).[51]

There are no reports on the clinical importance of WNV infection in cattle. Cases of WNF in cattle are rarely documented and serologic findings of WNV-specific IgG antibodies have been reported in other countries like Nigeria. In the Nigeria study, WNV IgG-antibody was documented in different animal sera including camels, goats, cattle and sheep.[52] WNV IgG-antibody was also reported in cattle in Senegal,[53] in Trinidad,[54] in Turkey,[55] and in Palestine.[56] A number of other studies in African countries have also reported anti- RVFV IgG and anti-WNV among apparently healthy animals,[57] including Kenya,[58,59] Tanzania,[58,60] Mozambique and Senegal.[49,51,59,61]

In Ethiopia in general and in Gambella in particular, there have been no reports of outbreaks or specific studies on RVFV and WNV in animals. Likely, this is due to the

Table 3 Seropositivity for WNV Infection in Cattle

Characteristics	Response Category	Total Tested	Sero IgG Status of WNV		P-value
			Negative No. (%)	Positive No. (%)	
Sex	Male Female	93 275	88(94.6) 260(94.5)	5 (5.4) 15(5.5)	0.384
Age group (N=368)	1–3.5 years 3.5+ years	222 146	208(93.7) 121(95.6)	14(6.3) 5(9.4)	0.720
Parity (N=275)	Yes No	121 154	116(95.9) 144(93.5)	5(4.1) 10(6.5)	0.432
Kebele	Pal Bol Kech Bilimkun Tandor	41 152 89 86	38(92.7) 143(94.1) 82(92.1) 85(98.8)	3(7.3) 9(5.9) 7(7.9) 1(1.2)	0.085
History of abortion (N=53 herds)	Yes No	27 26	24(88.9) 25(96.2)	3(11.1) 1 (3.8)	0.041
History of mass death in your animals (N=53 herds)	Yes No	23 30	21(91.3) 29(9.3.7)	2 (8.7) 2(6.7)	0.185
Previous signs and symptoms of excess salivation in sampled animals (N=368)	Yes No	52 316	50(96.1) 298(94.3)	2(3.9) 18(5.7)	0.249
Previous history of loss of appetite, weakness, diarrhea and excess nausea in sampled animals (N=368 sampled animals)	Yes No	96 272	91(94.8) 257(94.5)	5(5.2) 15(5.5)	0.560
All		368	348(94.6)	20(5.4)	

lack of virus screening measurements for the surveillance. Asymptomatic or unnoticed or mild form of the diseases may contribute to under-reporting of cases. However, few reports have documented RVFV and WNV infections in apparently healthy cattle.[48,62]

Many East African countries depend on pastoral agriculture for milk and meat production. Therefore, livestock diseases, including RVF may impact international livestock marketing and may harm the economics of a country like Ethiopia which depends on mainly on the livestock besides the direct impact on the human health.[63]

The current study showed the presence of RVFV and WNV-specific IgG antibodies and did not measure the level of anti-IgM antibodies, which would be an indicator of acute infection. Thus, this study cannot confirm recent infection, and the results may show only the exposure rate in the area or antibody cross-reactivity due to other arbovirus infections. The high IgG seropositivity to RVFV and WNV infections among the age group of 3.5+ years may show that these viruses have been occurred in the study area. However, WNV seroprevalence seems to have increased with time compared to RVFV because seropositivity in the age groups of 1–3.5 years was higher in the former than in the same age groups in the latter, where relatively higher seropositivity was observed among the age groups of 3.5+ years.

The seropositivity and sex of the cattle showed no significant association indicating that both males and females are affected by RVFV and WNV. However, male cattle were more likely affected by WNV infection than females, while the opposite was true for RVFV infection. Further studies are necessary to look at the reason for this result.

Other factors such as age and sampling sites did not significantly affect the occurrences of both viruses. In contrast to the present study, other studies have found a significant association between seropositivity of these viruses and two factors (age and sex).[13,60,64,65] For example, a study conducted in Madagascar reported higher prevalence of RVFV in male cattle compared to female

Table 4 Univariate and Multivariate Analysis of Risk Factors for RVFV Seropositivity

Characteristics	Response Category	Seropositive Status of RVFV IgG	
		Crude Odds Ratio (COR) (95% CI)	Adjusted Odds Ratio (AOR) (95% CI)
Sex	Male Female	0.69(0.30–1.60) 1	0.79(0.22–1.54) 1
Age group (N=368)	1–3.5 years 3.5+years	1 1.15(0.53–2.51)	1 1.47(0.32–6.82)
Parity (N=275)	Yes No	0.87(0.27–1.75) 1	0.97(0.19–4.91) 1
Kebele	Pal Bol Kech Bilimkun Tandor	1 1.42(0.36–5.62) 1.33(0.30–5.84) 0.49(0.14–1.83)	1 1.48(0.36–6.05) 1.40(0.30–6.42) 0.52(0.13–2.09)
Previous signs and symptoms of excess salivation in sampled animals (N=368)	Yes No	0.57(0.22–1.49) 1	0.45(0.14–1.40) 1
Previous history of loss of appetite, weakness, diarrhea and excess nausea in sampled animals (N=368 sampled animals)	Yes No	1.32(0.52–3.36) 1	1.68(0.54–5.18) 1

Abbreviations: COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence interval.

Table 5 Univariate and Multivariate Analyses of Risk Factors for WNV Seropositivity

Characteristics	Response Group	Seropositive Status of WNV IgG	
		COR (95% CI)	AOR (95% CI)
Sex	Male Female	1.02(0.36–2.87) 1	1.21(0.39–3.73) 1
Age group (N=368)	1–3.5 years 3.5+years	1 0.64(0.24–1.70)	1 0.61(0.10–3.84)
Parity (N=275)	Yes No	1.5(0.532–4.23) 1	1.07(0.15–7.61) 1
Kebele	Pal Bol Kech Bilimkun Tandor	1 1.25(0.32–4.86) 0.93(0.23–3.77) 6.71(0.68–66.62)	1 0.13(0.01–1.38) 0.18(0.02–1.44) 0.14(0.02–1.14)
Previous signs and symptoms of excess salivation in sampled animals (N=368)	Yes No	1.51(0.34–6.71) 1	1.57(0.29–8.50) 1
Previous history of loss of appetite, weakness, diarrhea and excess nausea in sampled animals (N=368 sampled animals)	Yes No	1.06(0.375–3.01) 1	0.86(0.24–3.03) 1

Abbreviations: COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence interval.

cattle.[50] In regards to the association of age-seropositivity of IgG antibodies in an endemic country, the occurrence of IgG antibodies in older animals generally supports the contention that most animals are exposed to the virus during their lifetimes. Infection of younger animals permits to pinpoint the time window of the infection and how recent the infection occurred.[50,60] Based on the multivariate logistic regression analysis, our study revealed 1.5

times higher seroprevalence of RVFV infection in the age group of 3.5+ years and a high seroprevalence of WNV infection in the age group between 1 and 3.5 years, which is in line with earlier studies showing a high proportion of IgG in similar age groups.[48,50,60] Enrolling a larger sample size might reveal a different association of the IgG result with age and sex.

Upon blood sample taking, livestock owners were also interviewed on some selected clinical signs related to RVFV infection since the WNF clinical signs in cattle have not been described. Almost half (50.9%) of the owners reported the occurrence of mass abortion in their herds, a finding significantly associated with RVFV seropositivity. Many (56.0%) of the livestock owners did not recall the history of the mass death of young animals. Nonspecific symptoms such as excess salivation were reported by 14.1% of respondents and loss of appetite, weakness, and diarrhea by 26.1% of participants together with signs of mass death of young animals and abortion. However, there was no significant difference between these variables and the serological documentation of WNV or RVFV infection. The statistical insignificance association of these signs and symptoms with outcomes of anti-RVFV and anti-WNV IgG, the signs and symptoms might arise due to other diseases.

In the present study, anti-WNV IgG-antibodies presence using ELISA was not confirmed by confirmatory to detect actual viral infection. Direct detection of the WNV infection from samples from various animal species would give an improved understanding of the epidemiological situation in east Africa. Serologically measured anti-WNV IgG antibodies in cattle in other African countries also support the possibility that infection causes clinical disease in these hosts like in birds, horses, crocodiles, and humans where many clinical signs shown. WNV occurrences in other species besides cattle elsewhere indicate the virus to be prevalent in birds, equine as well as in human populations.[66] The result of this study suggests the possible circulation of WNV and warrants performing further studies in Ethiopia with other species.

Limitations of the Study

In this study, the screening of the viruses with ELISA test was not supported with more sensitive and specific confirmatory tests like reverse transcription-polymerase chain reaction (RT-PCR). Another setback of this study is that it was conducted only on cattle, excluding other species of livestock in the study that would enable the comparison of the viruses' distributions in the available potential hosts. Finally, this study could not present antibody titers of the positive samples that might explain the results in advance. Cattle are the main means of economic importance in the area among the livestock population where pastoralists mainly depend on. In line with their importance, this is the first serologic study in cattle in the area which gives evidence of WNF and RVF presence, and encourages further studies. Besides, we believe that our study would provide baseline data on the epidemiology of WNF and RVF in the area.

Conclusions

The present antibody-based ELISA study revealed the possible occurrence of RVF and WNF among the cattle population in the study area. Significantly high IgG antibodies to both viruses was observed among those with a history of abortion. An increased trend of IgG-antibody positivity was also observed among animals in the age 3.5 years and above. Henceforth, the results warrant further studies toward active case detection of antigens of these viruses in susceptible hosts, including other livestock animals and humans, their transmission dynamics and their public health significance to predict and reduce possible future outbreaks in the area.

Abbreviations

RVFV, Rift Valley fever virus; RVF, Rift Valley fever; WNF, West Nile fever; WNV, West Nile virus; RT-PCR, reverse transcriptase-polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; PRNT, plaque reduction neutralization test; IgG, immunoglobulin G; IgM, immunoglobulin M; OD, optical density; RNA, ribonucleic acid.

Data Sharing Statement

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

All authors made a significant contribution to this work, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all

these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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RESEARCH ARTICLE

Seroprevalence of Yellow fever, Chikungunya, and Zika virus at a community level in the Gambella Region, South West Ethiopia

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Abstract

Yellow fever (YF), Chikungunya (CHIK), and Zika(ZIK) are among re-emerging arboviral diseases of major public health concern. Despite the proximity of the Gambella Region to South Sudan where arboviral cases have been recorded repeatedly the current epidemiological situation is unclear in this part of southwest Ethiopia. Therefore, we conducted a community-based seroprevalence survey of YF virus (YFV), CHIK virus (CHIKV), and ZIK virus (ZIKV) infections in two selected districts. A cross-sectional study was conducted in two locations of the Gambella region (Lare and Itang) to investigate the seroprevalence of these viruses' infections. Blood samples were collected from the study participants and screened for IgG antibodies specific to YFV and CHIKV infections using enzyme-linked immunosorbent assays (ELISA). For the detection of ZIKV specific IgG antibodies, Blockade-of-binding ELISA was used. Data were analyzed using the STATA version 13.1 Softwares. A total of 150 individuals (96 males and 54 females, age ranging from 18 to 65 years, mean age ± SD = 35.92 ± 10.99) participated and provided blood samples. Among the 150 samples 135, 90, and 150 were screened for YFV, CHIKV, and ZIKV, respectively. Hence, 2.9% (95% CI: 1.1–7.7%), 15.6% (95% CI: 9.3–24.8%), and 27.3% (95% CI: 20.7–35.3%) of samples tested positive for IgG antibodies to YFV, CHIKV, and ZIKV infections, respectively. Among the individual seropositive for ZIKV, YFV and CHIKV, only six, one and three had a history of residence outside the Gambella region respectively. Agro-pastoral occupation was significantly associated with a higher prevalence of IgG against CHIKV (AOR = 14.17; 95%CI: 2.30, 87.30) and residency in the Lare district (AOR = 11; 95%CI: 3.31, 39.81) was found to be significantly associated with a higher prevalence of IgG against ZIKV. Our findings revealed the occurrence of YFV, CHIKV and ZIKV infections in the study locations.

analysis, decision to publish, or preparation of the manuscript.

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Introduction

Emerging and re-emerging mosquito-borne viruses such as Yellow fever virus (YFV), Chikungunya virus(CHIKV), Dengue virus, and Zika virus (ZIKV) have become major public health concerns in tropical and subtropical countries [1–3]. Climate change, vector adaptations, urbanization, migration of people, and expansion of agricultural activities to sylvatic areas are among the factors contributing to the spread of arboviruses to a wider range of geographical areas [4].

YFV is a re-emerging arbovirus that belongs to the genus *Flavivirus* [5]. YFV is transmitted by several mosquitoes species (spp) including *Aedes* spp., *Haemagogus* spp., and *Sabethes* spp [6]. Africa accounts for ~90% of the global YFV infection cases. YF is an endemic and intermittently epidemic disease affecting a wide range of the continent [7]. Recently, African countries like Nigeria [7], Uganda [8], Ghana, Chad, Guinea, the Republic of the Congo [8], and Angola [9] reported YF outbreaks. In Ethiopia, YF outbreaks have repeatedly occurred since the 1960's resulting in over 30,000 deaths in the southern part of the country [10]. In 2013, a YF outbreak re-occurred in the South Omo Zone of southern Ethiopia resulting in many deaths [3]. Recently, another YF outbreak was reported from the Gurage and Wolayita areas of southern Ethiopia [11].

CHIKV of the genus *Alphavirus* is transmitted through the bite of an infected female mosquito belonging mainly to *Ae*. *aegypti* and *Ae*. *albopictus*. CHIK is a zoonotic disease widely distributed in many tropical and subtropical regions of sub-Saharan Africa [12, 13] including Sudan [14], Kenya [15], Tanzania [16], and Uganda [17]. In June 2016, Ethiopia confirmed its first documented case of CHIK from the Suuf kebele, Dollo Ado district of the Somalia regional state of Ethiopia bordering the Mandera region of Kenya, where a CHIKV outbreak was ongoing [18, 19]. Additionally, CHIK cases were reported from the Dire Dawa and the Afar regions, Eastern Ethiopia [20, 21]. Recently, a study by Endale and his co-authors reported a high seroprevalence (43.6%) of CHIKV infections in the South Omo region of southwest Ethiopia which adjoins the current study location (Gambella region, Southwest Ethiopia) [22]. There is no data about CHIKV circulation in the Gambella region.

ZIKV is a *Flavivirus* first isolated in 1947 from a rhesus monkey resident in the Zika forest of Uganda [23]. ZIKV infection of humans was first reported from Uganda and Tanzania in 1952 [24]. Like CHIKV, ZIKV transmission occurs via the bite of an infected female mosquito belonging mainly to *Ae*. *aegypti* and *Ae*. *Albopticus* [25]. Recent reports confirmed sexual transmission of ZIKV and transmission through blood transfusion [26], and also vertical transmission from mother to the fetus [27]. There is no data about ZIKV circulation in the Gambella region.

The Gambella Region has been selected as a study site because it is adjacent to the South Omo area in Ethiopia where YF is endemic [3]. This region is also proximal to South Sudan and Kenya where many arboviral cases are frequently reported [28–30]. Besides the geographical proximity of the area, the risk of introduction of disease is expected to increase due to the free movement of domesticated animals, wild game, and migration of people between these areas.

No epidemiologic information on arboviral diseases in the Gambella region is available, due to the lack of community- or health facility-based studies. We now report the first community-based seroprevalence study of YFV, CHIKV, and ZIKV infections in the Gambella region in the South West of Ethiopia.

Materials and methods

Study area and population

This study was conducted in the Gambella Region of South West Ethiopia between late October 2018 and mid-June 2019. Gambella is located at an elevation between 1,000 to 2,000 meters

above sea level (masl) in the East, to 500–900 masl in the center, and 300–500 masl in the West [31] with a recent population projection estimated to be 435,999 [32]. Lare and Itang special districts were selected as study sites for this research. Lare is located at 300–500 masl in the West and borders South Sudan. Itang's special district borders are Lare and South Sudan to the West. These districts were purposely selected because of their proximity to neighboring countries like South Sudan and Kenya where outbreaks of different arboviruses have been reported [33, 34]. Specific sub-districts were also considered during selection based upon their proximity to refugee camps, as well as the ecology of the area such as forest, water bodies, population density, and the occurrence of mosquito vectors and prevalence of arboviral infections in the bordering area.

These districts host many refugees from South Sudan and migratory pastoralists from South and North Sudan (commonly called "Fallata" or "Fulani"). In addition, native pastoralists of the Lare district traditionally travel far from their villages to the adjacent territories of South Sudan for social activities and in search of pasture. In this study, six "kebeles" (the lowest administrative structure in a district in Ethiopia) from the Itang special district, which are closer to the refugee camps, and four "kebeles" from Lare district, contingent to the border of South Sudan were included (Fig 1).

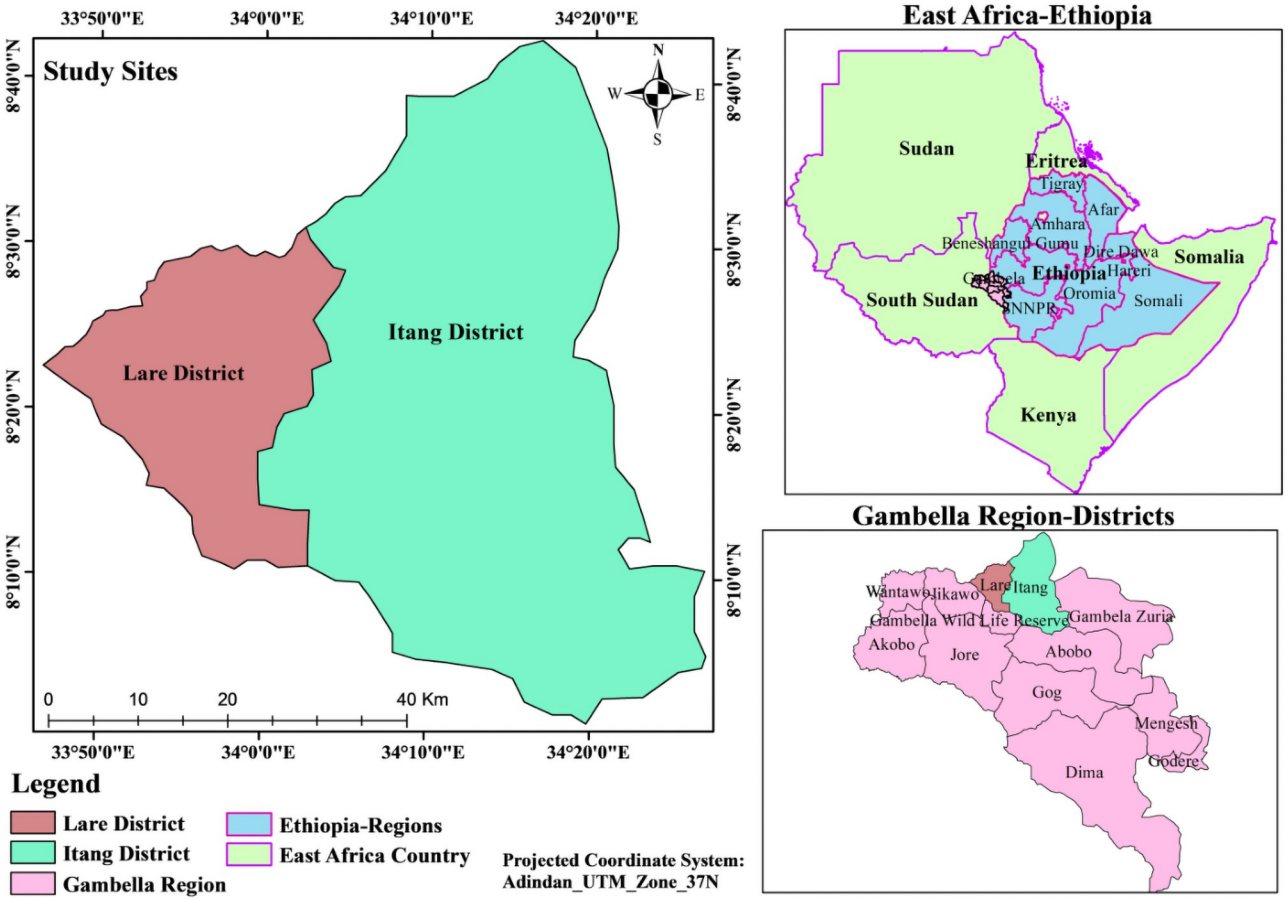

Fig 1. Map of the study site (Lare district and Itang special districts).

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Study design, sample size, and sampling techniques

A community-based volunteer study was designed. Inclusion criteria were: age ≥18 years, ability to give written consent and respond to the questionnaire, known resident in the "kebele" for more than six months, apparently healthy (with no current illness, not suffering from obvious medical and psychiatric problems), and able to provide 3 ml blood sample. The locations of and proximity to the refugee camps, community movements with bordering territories, and the adjacent sub-districts were documented. Pregnant women, sick individuals, refugees, and visitors to the area were excluded from the study. Refugees were excluded because the antibodies detected in these vulnerable groups may have resulted from a previous infection in their country of origin and would certainly not measure exposure to the disease in the study area. During the sampling procedures, sub-districts were purposively selected seeking areas with a higher mosquito burden.

One hundred fifty (150) community members gave written consent and provided a 3 ml venous blood sample once during the study period.

All 150 blood samples were screened for IgG specific to ZIKV using the blockade-of-binding (BOB) technique. Blood samples from 135 for YFV and 90 participants were also screened for IgG specific to YFV and CHIKV, respectively, using indirect enzyme-linked immunosorbent assay (ELISA) techniques.

Data collection and laboratory investigation

Data including socio-demographic characteristics, duration of stay in the study area, history of residence/travel in other countries, and history of vaccination against YFV or other arboviruses were collected using a structured questionnaire (S1 and S2 Questionnaires). Venous blood samples of each study participant were collected using serum separator vacutainer test tubes uniquely labelled for each person. To increase the amount of serum and maximum separation the test tubes were centrifuged for 15 minutes at 3400 rotations per minute, and the serum separated and stored at -20˚c until screened for immunoglobulin G (IgG) antibodies against YFV and CHIKV using a sandwich ELISA assay (Abbexa Ltd, Cambridge UK) [35] as described in detail by the manufacturer. In brief, a 96 well plate was pre-coated with the target antigen. Two of the wells were then aliquoted with 50μl of the negative and positive controls into the set wells, respectively. One well was left as the control (zero) blank. Similarly, we aliquoted 50μl appropriately diluted samples into the test sample wells with a dilution rate of 1/5. The solution was added at the bottom without touching the sidewalls of the well and the plate was shaken gently to mix the contents. The controls and test samples are incubated at 37˚C for 30 minutes after sealed with a ready-made cover. After 30 minutes incubation, the cover was removed and the plate washed 5 times with buffer solution. Then 50μl of Horseradish Peroxidase (HRP) conjugate reagent was added to each well (except the blank well) and sealed again for incubation at 37˚C for 30 minutes. The plates were then washed with buffer five times and aliquot 50μl of Tetramethyl benzidine (TMB) substrate A into each well, 50μl of TMB Substrate B added. Then plate was shaken gently by hand for 30 seconds, covered and incubated at 37˚C for 15 minutes avoiding exposure to light. The HRP catalyzes TMB to produce a blue color product that changes to yellow after adding the acidic stop solution. The intensity of the yellow color is proportional to the YFV-IgG/CHIKV-IgG bound on the plate. The Optical density (OD) absorbance is measured spectrophotometrically at 450nm in a microplate reader, and the presence of YFV-IgG/ CHIKV-IgG is determined [35].

Each serum was also screened for IgG antibodies against ZIKV using the ZIKV NS1 BOB assay as previously developed in Nicaragua [36]. This is highly specific for ZIKV with minimal to no cross-reactivity to other flaviviruses. In brief, a 96 well plate is coated overnight with

recombinant purified 1 ug/ml ZIKV NS1 Uganda strain (Native antigen) protein and blocked with PBS 1% bovine serum albumin for 1 h. The plate is then incubated with 1:10 diluted serum from each study participant for 1 hour and then HRP-labelled anti-ZIKV NS1 (mAB ZKA35, Absolute antibody) diluted 1:5000 in PBS with 1% BSA is added to each well and incubated for another 15 min. The plate is then washed and TMB substrate (Sigma) added to each well and incubated for 5–6 min in the dark and the reaction is stopped with 2N H_2SO_4 or 1N HCL. The plate is read on a plate reader (Multiskan™ FC Microplate Photometer) at an absorbance of 450 nm. Unconjugated ZKA35 rIgG1 (1:200 diluted) is added as a positive control and normal human serum (NHS) is added as a negative control (1:10 diluted). The percentage of ZKA35-HRP binding inhibition is calculated as described in detail by Balmaseda and his colleague [36]

The concentration of ZKA35-HRP used in the BOB assay corresponds to 70% of the maximal OD (450 nm) level as determined by interpolating a curve fitted with a 4-parameter non-linear regression. A starting dilution at 1:10 and then 1:3 serial dilution of 12 points in assay diluent is performed using 50 μl ZKA35-HRP/well.

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board (IRB) of the Aklilu Lemma Institute of Pathobiology, Addis Ababa University. Permission to visit the study sites and to collect the blood samples was obtained from the Gambella Regional Health Office, district administration offices, and community leaders of each study site. The objective of the study was explained to each of the study participants and written consent was obtained from each participant. Finally, blood sample collection was carried out under aseptic conditions by experienced medical laboratory technicians.

Statistical analysis

Collected data were coded and entered into Epi Data Software v.3.1 and analyzed using STATA 13.1. Socio-demographic characteristics were summarized using the frequencies and percentages while the seroprevalence of IgG antibodies elicited towards YFV, CHIKV, and ZIKV were calculated by dividing the number of participants with positive test results by the total number of study participants. The associations between the seroprevalence of IgG and the background characteristics such as age, sex, occupation, education, traveling/residency history in other countries or areas outside Gambella were assessed using the uni-variable logistic regression analysis. The effects of each independent variable (i.e. gender, age, occupation, ethnicity, education, and others) on the outcome variable after adjusting each independent variable for all other variables were analyzed using the multivariable logistic regression. The coefficient values of each independent variable (predictors) are included in the final reported model. All analysis results with a P-value below 0.05 were considered statistically significant.

Results

Socio-demographic characteristics of study participants

The socio-demographic characteristics of all study participants are summarized in Table 1. A total of 150 individuals (96 males and 54 females, age ranging from 18–65 years) from two districts (33 from Lare and 117 from Itang special district) participated in this study. Most travelers were in the 18–30 age group (17.5%). The travel or living /residency history outside Gambella was higher in males (16.7%) than females (7.4%) as shown in Fig 2 (P-value>0.05).

Table 1. Socio-demographic characteristics (N = 150).

Variables	Response category	Travel history		History of working in the forest	
		Yes N(%)	No N(%)	Yes N(%)	No N(%)
Sex	Male	16(16.7)	80(83.3)	79(82.3)	17(17.7)
	Female	4(7.4)	50(92.6)	47(87.0)	7(13.0)
District	Itang	15(12.8)	102(87.2)	96(82.1)	21(17.9)
	Lare	5(15.2)	28(84.8)	30(90.9)	3(9.1)
Age (years)	18–30 years	10(17.5)	47(82.5)	48(84.2)	9(15.8)
	31–40 years	5(10.2)	44(89.8)	40(81.6)	9(18.4)
	≥41 years	5(11.4)	39(88.6)	38(86.4)	6(13.6)
Ethnicity	Nuer	15(15.5)	82(84.5)	92(94.8)	5(5.2)
	Agnewak	5(9.4)	48(90.6)	34(64.2)	19(35.8)
Education level	Informal	9(9.7)	84(90.3)	83(89.3)	10(10.7)
	Formal	11(19.3)	46(80.7)	43(75.4)	14(24.6)
Occupation	Pastoralist	8(13.8)	50(86.2)	53(91.4)	5(8.6)
	Agro pastoralist	6(16.7)	30(83.3)	35(97.2)	1(2.8)
	Others	6(10.7)	50(89.3)	38(67.9)	18(32.1)
History of vaccination	yes	0(0.0)	0(0.0)	-	-
	No	20 (13.3)	130(86.7)	126(84.0)	24(16.0)
History of bite by *Aedes* mosquito	Yes	17(14.4)	101(85.6)	105(89.0)	13(11.0)
	No	3(9.4)	29(86.6)	21(65.6)	11(34.4)

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In the Lare district 15.2% of the participants had traveled or lived outside of Gambella (P-value >0.05). None of the study participants responded positively regarding YF vaccination.

Seroprevalence and independent predictors of sero-positivity for YFV infection

Out of the 135 randomly screened serum samples for IgG antibodies to YFV, only 4 samples (2.9%; 95% CI: 1.1–7.72%) were found to be positive (Table 2). All the YFV-IgG positive individuals were from the Itang district; hence site/district was omitted during logistic regression analysis due to zero outcomes from the Lare district. There were no identified risk factors significantly associated with seropositivity for specific IgG antibodies to YFV. However, there were higher proportion of males (3.6%) and individuals in the age group of 31–40 years (5%) having YFV specific IgG antibodies compared to the females (2%) or other age categories, respectively (Table 2). Participants with a history of residence/travel outside Gambella (AOR = 3.10, 95%CI: 0.21–44.56%) and those with an occupation engaged in agro-pastoralist (AOR = 1.92, 95% CI: 0.11–35.05%) were more likely to be IgG positive for YFV infection albeit not statistically significant. Participants with a history of a bite by *Ae* mosquitoes, and working in the forest showed protective odds in the contrary (Table 2).

Seroprevalence and independent predictors of sero-positivity for CHIKV infection

Out of the 90 samples 14 (15.6%, 95% CI: 9.3–24.8%) were positive for IgG antibodies against CHIKV (Table 3). In the multivariable logistic regression analysis model, a 14-fold lower sero-positivity for CHIKV specific IgG was detected among pastoralists compared to agro-pastoral (AOR = 14.17; CI: 2.30, 87.30) (Table 3). A higher proportion of anti-CHIKV IgG antibodies

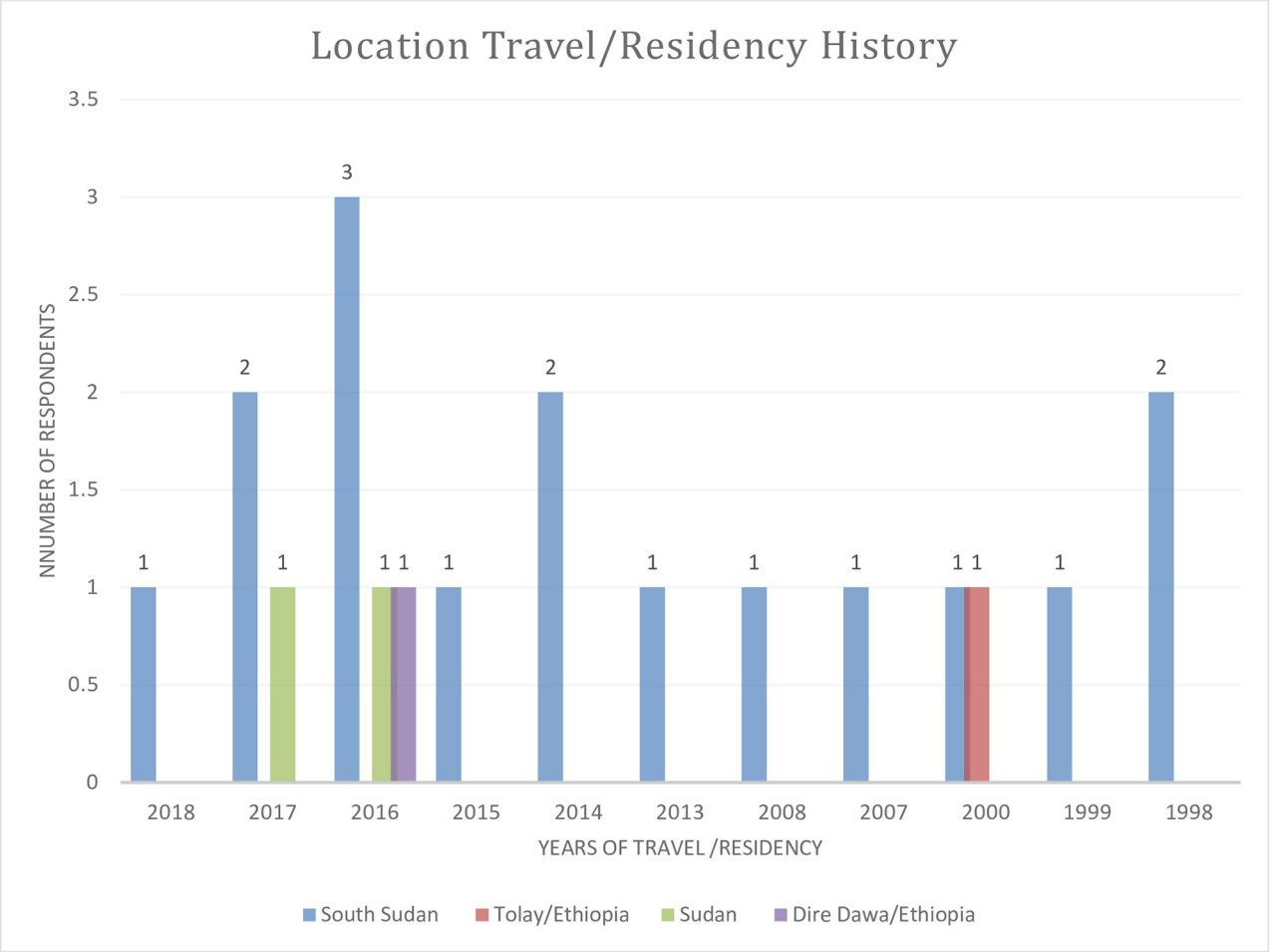

Fig 2. Traveling and residence out of Gambella region among those with a history of the travel.

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was observed in the age group ≥41 compared to younger age categories although not statistically significant.

Seroprevalence and independent predictors of sero-positivity for ZIKV infection

Out of the 150 samples tested for the presence of IgG antibodies against the ZIKV, 41 (27.3%; 95% CI: 20.7–35.1%) were found to be positive (Table 4). Multivariable logistic regression analysis showed that living in Lare (AOR = 11.5; 95% CI: 3.31–39.81%), being a female (AOR = 4.8; 95% CI: 1.62–14.63%) and being a pastoralist (AOR = 5.1; 95% CI: 1.44–17.80%) were significantly associated with seropositivity for ZIKV.

Discussion

Arboviral infections and outbreaks in Ethiopia have been documented since the 1960s, yet the present epidemiological situation in the country remains unknown [37]. This is the first sero-epidemiological study of YFV, CHIKV, and ZIKV infections in community members from the Gambella regional state of Ethiopia.

Table 2. Sero-positivity for IgG antibody to YFV infection and associated factors (N = 135).

Variables	Category	YF IgG sero-status		COR (95% CI)	AOR (95% CI)
		Total tested(N)	Positive N (%)		
Sex	Male	84	3 (3.6)	1.85(0.19, 18.30)	1.32(0.10, 17.03)
	Female	51	1(2.0)	Ref	Ref
Age Group (years)	18–30	53	1(1.9)	0.79(0.05, 12.99)	0.77(0.03, 17.64)
	31–40	40	2(5.0)	2.16(0.19, 24.77)	2.29(0.17, 31.61)
	≥41	42	1(2.4)	Ref	Ref
Educational Status	Formal education	51	2(3.9)	1.7(0.23, 12.26)	1.31(0.10, 17.23)
	Informal education	84	2(2.4)	Ref	Ref
Occupation	pastoralist	55	1(1.8)	Ref	Ref
	Agro pastoralist	32	1(3.1)	1.74(0.10,28.84)	1.92(0.11, 35.05)
	Others	48	2(4.2)	2.35(0.21, 26.73)	1.18(0.05,25.41)
History of residence/travel outside Gambella	Yes	17	1 (5.9)	2.4(0.23, 24.45)	3.10(0.21, 44.56)
	No	118	3 (2.5)	Ref	Ref
History of working in the forest areas	Yes	113	3(2.6)	0.57(0.06, 5.77)	0.47(0.03, 7.68)
	No	22	1(4.5)	Ref	Ref
History of Bite by *Aedes* mosquito	Yes	109	3(2.7)	0.54(0.05, 5.44)	0.74(0.04, 12.25)
	No	26	1(3.85)	Ref	Ref
District	Itang	103	4(3.9)	-	-
	Lare	32	0(0.0)	-	-
History of vaccination	Yes	0(0.0)	0(0.0)		
	No	135	4(2.9)	-	-

CI (confidence interval), COR (crude odds ratio), and AOR (adjusted odds ratio), Ref (Reference category).

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Our study examines the community-based sero-epidemiology of YFV, CHIKV, and ZIKV in two selected districts of the Gambella region in South West Ethiopia. Previously a few studies on arboviruses have been conducted in other regions of Ethiopia and were mainly part of outbreak investigations at that time including dengue fever (DF) and YF [3, 19, 22, 37, 38].

We found a seroprevalence of 2.9% of YFV specific-IgG in collected blood samples.

Of the four YFV IgG-positive individuals, three had no history of residency or travel outside the Gambella region, thus indicating that YFV infections occurred in this area.

Besides, three of the four individuals reported a history of working in the forest area for farming, where they might have been exposed to mosquito bites. Indeed arboviruses commonly circulate in the forest area in a sylvatic cycle involving primates as reservoir hosts [13]. The prevalence rate of IgG antibodies against YFV in the Gambella region is in line with the seroprevalence detected in a previous study conducted in Djibouti (14 ELISA positive out of 903 screened) [39]. However, another study from another part of Ethiopia revealed that the seroprevalence of YFV among study participants was 0.6% in pooled samples [19]. The slightly higher seroprevalence that we found in the Gambella region could be explained by the higher rate of daily commuters from neighboring countries (South Sudan, Sudan), migration of refugees, and seasonal movement of pastoralists from North and West Africa in search of pasture. Our findings show a lower anti-YFV IgG antibody response compared to other seroprevalence studies conducted in South Omo, Ethiopia [22] where the authors found 49.5% seropositivity for YFV IgG antibodies. A higher seroprevalence of YFV IgG antibodies was also reported from the Borena area of Southern Ethiopia (12.5% of participants) [40], and Kenya (6% of participants) [41]. These variations might be due to different sample sizes, and higher vaccination

Table 3. Seropositivity for IgG antibody to CHIKV and associated factors (N = 90).

Variables	Category	CHIKV IgG sero-status		COR (95% CI)	AOR (95% CI)
		Total tested (N)	Positive N (%)		
Site	Itang	58	12(20.7)	Ref	Ref
	Lare	32	2(6.3)	0.25(0.05, 1.22)	0.43(0.06,2.82)
Sex	Male	53	12(22.6)	5.12(1.07, 24.46)*	3.78(0.58, 24.52)
	Female	37	2(5.4)	Reference	Reference
Age Group (years)	18–30	36	4(11.1)	0.48(0.12, 1.89)	0.75(0.11, 5.37)
	31–40	25	4(16.0)	0.73 (0.18, 2.95)	2.74(0.47, 16.08)
	≥41	29	6(20.7)	Ref	Ref
Educational Status	Informal education	64	8(12.5)	Ref	Ref
	Formal education	26	6(23.1)	2.1(0.65, 6.80)	2.64(0.36, 19.29)
Occupation	Pastoralist	49	2(4.1)	Ref	Ref
	Agro pastoralist	26	9(34.6)	12.44(2.44,63.47)*	14.17(2.30, 87.30)*
	Others	15	3(20.0)	5.88(0.88, 39.21)	2.20(0.17, 27.18)
History of residence outside Gambella	Yes	10	3(30.0)	2.68(0.60, 11.98)	2.44 (0.33, 17.69)
	No	80	11(13.7)	Ref	Ref
History of working in the forest areas	Yes	82	13(15.8)	1.32(0.14, 11.64)	0.90(0.05, 15.33)
	No	8	1(12.5)	Ref	Ref
History of Bite by *Aedes* mosquito	Yes	89	12(15.2)	0.54(0.10, 2.98)	0.73(0.1, 8.00)
	No	11	2(18.2)	Ref	Ref

CI (confidence interval), COR (crude odds ratio), AOR (adjusted odds ratio), and
*(significant at $p<0.05$), Ref (Reference category).

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Table 4. Seropositivity for IgG antibody to ZIKV and associated factors in the study participants (N = 150).

Variables	Category	ZIKV IgG sero-status			COR (95% CI)	AOR (95% CI)
		Total tested N (%)	Positive N (%)	Equivocal N (%)		
Site	Itang	117	15(12.8)	2(1.7)	Ref	Ref
	Lare	33	26(18.8)	0(0.0)	21.8(8.20, 58.23)*	11.5(3.31, 39.81)*
Sex	Male	96	15(15.6)	1(1.0)	Ref	Ref
	Female	54	26(48.2)	1(1.8)	5.0(2.34, 10.65)*	4.8(1.62, 14.63)*
Age Group (years)	18–30	57	23(40.3)	0(0.0)	2.1(0.90,4.8)	1.5(0.39, 5.61)
	31–40	49	11(22.5)	1(2.0)	1.5(0.53, 4.0)	0.80(0.21,3.13)
	≥41	44	7(15.9)	1(2.3)	Ref	Ref
Educational Status	Informal education	93	32(34.4)	1(1.1)	2.59(1.16, 5.76)*	1.0(0.28,3.68)
	Formal education	57	9(15.8)	1(1.8)	Ref	Ref
Occupation	Pastoralist	58	32(55.2)	1(1.7)	8.2(2.78, 24.1)*	5.1(1.44, 17.80)*
	Others	56	5(8.9)	0(0.0)	0.61(0.16, 2.27)	1.5(0.27,8.22)
	Agro pastoralist	36	4(11.1)	1(2.8)	Ref	Ref
History of residence /travel outside Gambella	Yes	20	6(30.0)	0(0.0)	1.10(0.38, 3.0)	1.4(0.29,6.65)
	No	130	35(26.9)	2(1.5)	Ref	Ref
History of working in the forest areas	Yes	126	36 (28.6)	2(1.6)	1.64(0.57, 4.72)	1.15(0.20, 6.49)
	No	24	5(20.8)	0(0.0)	Ref	Ref
History of bite by *Aedese* mosquito	Yes	118	36(30.5)	2(1.7)	5.7(1.28, 25.37)*	1.34(0.21, 8.47)
	No	32	5(15.6)	0(0.0)	Ref	Ref

CI (confidence interval), COR (crude odds ratio), AOR (adjusted odds ratio), and
*(significant at $p<0.05$), Ref (Reference category).

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history in the population. No YF vaccinations was recorded in any of our subjects at both study sites. The comparison of different studies is difficult due to differences in methodology, diagnostic tools, and characteristics of the study populations. For instance, the current study focused on the detection of IgG antibodies in blood samples of healthy donors using an ELISA while others implemented advanced diagnosis methods. In addition, other groups used sera from individuals with suspected infection rather than from an apparently healthy community. IgG antibodies against YFV were also observed in a study conducted in Kenya among subjects between 30–40 years of age [42].

Among the 14 individuals having anti-CHIKV IgG, only three had a history of residency or travel out of Gambella. This might indicate that CHIKV infections occurred in the study area but detection of IgM or viral RNA is necessary to confirm current virus circulation. Almost all seropositive individuals were engaged in farming activities in the forest area and had reported mosquito bites. The prevalence of anti-CHIKV IgG in our study (seroprevalence 15.6%) is lower than in Sudan, Kassala (73.1%) [43], where only patients suffering from unknown fever were tested. In contrast, Khartoum state in Sudan and Southern Mozambique reported a low seroprevalence of CHIKV IgG antibodies of 2.2% [44] and 4.3% [45], respectively among febrile patients. Thus, these studies differ from the present study in Gambella, where we screened only healthy subjects. The majority of the CHIKV IgG+ cases in our study were between 31–40 years of age, which is similar to findings of a seroprevalence study in Tanzania [46]. The highest IgG seroprevalence of CHIKV was found in males which was fivefold (22.6%) higher compared to females, supporting the contention that males are more exposed to mosquito bites during farming activity or other similar travel or working related factors. These findings are in agreement with another study that showed that males are more susceptible than females [47, 48]. However, other seroprevalence studies observed the opposite trend, where females were more often CHIKV IgG+ compared to males [49, 50]. The detection of IgG against CHIKV was significantly associated with the agro-pastoralist lifestyle compared to a pastoralist one, which could be associated with the job-related risk including environmental suitability for vector and maintenance of the virus in such conditions as well as the intimate contact between humans, and primates. The discrepancy with results from other studies might be due to sampling size and proportions of the various categories such as gender and age. Furthermore, the participating community could have various habits, behaviors, occupations, traditions, and local practices that would expose them to the virus more or less likely.

In the current study, there is serological evidence of human exposure to ZIKV in the two districts of the Gambella region, Southwest Ethiopia. Antibodies against ZIKV were found in 41 individuals (27.3%; 95% CI: 20.7, 35.1%). Among those 41, only six individuals reported a history of traveling or residing outside of Gambella. Almost all individuals reported visiting forest areas for their farming activities, where they might have been infected via mosquitoes which are in in close contact with the reservoir hosts such as primates. Indeed, most of the positive individuals had reported mosquito bites. Markedly, the seroprevalence of anti- ZIKV IgG found in Gambella was higher than studies conducted elsewhere in Ethiopia in 2018 (0.4%) [19], in Kenya (3.9%) [43], and Zambia (6.1%) [51], but was similar to the seroprevalence reported in Nigeria (31%) [52]. Our study confirms that ZIKV infections have occurred in the study area of Ethiopia. A large fraction of the ZIKV IgG+ cases were found in the Lare district (11 fold higher than Itang special district), which could be due to Lare being the main entry point from South Sudan to Ethiopia, and is a temporary residential location and a registration place for refugees from South Sudan. Neighboring countries such as Kenya have also reported a high seroprevalence of ZIKV IgG+ antibodies in many region [53, 54]. Significantly higher ZIKV IgG+ antibodies were found in females in this study which is similar to a study conducted elsewhere [55]. Studies indicated that females in the sexually active age group are more

likely to get ZIKV than males; sexual transmission is the most probable cause [56]. A gender-specific prevalence of ZIKV is also observed in mouse models [57] as female mice are more affected. In contrast to this study's finding, a high risk of ZIKV infection in males was observed in Kenya [42] even if not significant. These differences might be explained by the variation in gender proportion, the difference in laboratory tests applied, presence of non-vector transmissions, and the traditions of the community which poses a predisposition factor for ZIKV infection. In general, a large fraction of the age groups 18–30 and 31–40 years was ZIKV IgG + compared to individuals in the age group ≥41 years, which is in line with a study result from Zambia where study participants between 24–44 years of age showed a higher trend of ZIKV IgG seropositivity compared to older participants [51]. This result could be related to differences in mobility, occupation, and immune status between age groups. Participants with a history of residence or travel outside of Gambella also showed a higher trend for being ZIKV IgG + which is in agreement with other study results [51, 58]. Participants who had a history of working in the forest areas were more likely to be ZIKV IgG+. Notably, pastoralists were found to be significantly more affected compared to agro-pastoralists which is in line with another study from Kenya [42]. This could be due to the tradition of the pastoralists and their lifestyle associated with long-distance movements with their livestock in search of pasture or water which exposes them more to forest areas and mosquito breeding sites close to water sources. Participants with informal education were more likely to have ZIKA IgG+ antibodies relative to those who have attended formal education. This might correlate with lower knowledge of disease transmission and the source of infection as shown by a study conducted in Nigeria [59].

Limitation of the study

One limitation of the study is that we did not use confirmatory tests such as Plaque Reduction Neutralization Test (PRNT) as proof of the viral agent due to budget limitations. In addition, we were unable to screen all the serum samples for YFV and CHIKV also due to budget limitations. Thus, the number of serum samples screened may be a limitation of the study. Finally, we did not conduct IgM ELISA screening to help distinguish recent exposure vs. more distant viral infection.

Conclusions

Our community-based seroprevalence study showed the circulation of YFV, CHIKV, and ZIKV in the two districts of the Gambella region of Ethiopia. We found that the seroprevalence of anti-CHIKV and anti-ZIKV IgG antibodies were significantly higher in the agro-pastoral and pastoralists communities respectively. Moreover, the seroprevalence of anti-ZIKV IgG was significantly higher in females and in individuals from the Lare district. Therefore, additional testing options are needed in local health facilities and laboratories to help implement mosquito-borne viral disease prevention and control programs.

Supporting information

S1 Protocol.
(DOCX)

S1 Questionnaire.
(DOCX)

S2 Questionnaire.
(DOCX)

S1 Data.
(XLS)

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