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EPIDEMIOLOGY OF RIFT VALLEY FEVER VIRUS AND WEST NILE VIRUS IN LIVESTOCK POPULATIONS AND THEIR PUBLIC HEALTH IMPLICATIONS IN THE AFAR REGION, NORTHEASTERN ETHIOPIA

PhD Dissertation

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College of Veterinary Medicine and Agriculture

Department of Epidemiology and Veterinary Public Health

PhD Program in Veterinary Public Health

May, 2025

Bishoftu, Ethiopia

EPIDEMIOLOGY OF RIFT VALLEY FEVER VIRUS AND WEST NILE VIRUS IN LIVESTOCK POPULATIONS AND THEIR PUBLIC HEALTH IMPLICATIONS IN THE AFAR REGION, NORTHEASTERN 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

By

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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 Jemberu Alemu titled: **Epidemiology of Rift Valley Fever Virus and West Nile Virus in Livestock Populations and Their Public Health Implications in the Afar Region, Northeastern 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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BIOGRAPHICAL SKETCH

The writer of this dissertation was born on September 28, 1987, in Mechimena, Meskan district of East Gurage zone, Ethiopia. He began his education at Gogetti Primary and Secondary School, where he completed his primary studies between 1995 and 2002. He then pursued his high school and preparatory education at Butajira, Ethiopia, from 2002 to 2006. In 2006, He joined the Faculty of Veterinary Medicine at the University of Gondar, where he graduated with a Doctor of Veterinary Medicine (DVM) degree on 09 July 2011.

Following his graduation, He embarked on a teaching career. In December 2012, he joined Mettu University, Gambella Agriculture and Natural Resource Faculty, as a Lecturer, and later that same year transferred to Gambella University. In pursuit of further academic excellence, He enrolled in Addis Ababa University, where he obtained an MSc in Veterinary Public Health on 09 July 2015.

After completing his postgraduate studies, He took on administrative roles at Gambella University. From December 2015 to September 11, 2017, he served as the Coordinator of the School of Graduate Studies. He was later appointed Dean of the School of Graduate Studies, serving from September 2017 to September 2019 for full term. Throughout his time at Gambella University, he actively participated in various committees, seminars, workshops, and local and international trainings, contributing to academic growth through research activities and community service initiatives. He also holds a Higher Diploma Program (HDP) Certificate of Competency for Teaching in Higher Education Institutions, earned during his tenure at Gambella University. In December 2020, He commenced his PhD study in Veterinary Public Health at Addis Ababa University, College of Veterinary Medicine and Agriculture.

Throughout his academic and professional career, he has authored more than 18 articles published in reputable local and international journals. These publications reflect his unwavering commitment to advancing veterinary science and education. His work has made significant contributions to research, community service, and leadership in higher education, solidifying his role as a key figure in Ethiopia's academic and scientific communities.

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ACKNOWLEDGMENTS

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I wish to express my deep appreciation and gratitude to the College of Veterinary Medicine and Agriculture, Addis Ababa University, Department of Epidemiology and Veterinary Public Health and Aklilu Lemma Institute of Health Research (AL-IHR) for the financial support, lab facility, and logistic services rendered during the study. I would also like to thank Gambella University for giving me the PhD study chance to pay my base salary during my study leave.

I would like to thank the staff of Aklilu Lemma Institute of Health Research (AL-IHR), Addis Ababa University and I am deeply grateful to AL-IHR Serology Laboratory staff specially Dr. Muse for his excellent support in laboratory work at AL-IHR. I also extend my gratitude to Mr. Sisay, Mr. Getachew and others for excellent support and safe driving during the fieldwork day and night to trace the livestock in pastoral communities.

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LIST OF ABBREVIATIONS

AL-IHR	Aklilu Lemma Institute of Health Research
AGID	Agar Gel Immunodiffusion
ANRS	Afar National Regional State
ARRIVE	Animal Research Reporting *In Vivo* Experiment
CDC	Center for Disease Control and Prevention
CFR	Case Fatality Rate
CFSPH	Center for Food Security and Public Health
CNS	Central Nervous System
CPE	Cytopathic Effect
CSA	Central Statistics Authority
DHCPP	Division of High-Consequence Pathogens and Pathology
EHNRI	Ethiopian Health and Nutrition Research Institute
ELISA	Enzyme Linked Immunosorbent Assay
ENSO	El Niño Southern Oscillation
EU	European Union
FAO	Food and Agricultural Organizations of the United Nations
FGD	Focus Group Discussion
GDP	Gross Domestic Product
HI	Hemagglutination Inhibition
ID-Vet	Innovative Diagnostics
IGAD	Intergovernmental Authority on Development
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IU	International Units
MoH	Ministry Of Health
Nc	Negative Control
NCEZID	National Center for Emerging and Zoonotic Infectious Diseases
NSP	Nonstructural Proteins
OD	Optical Density

OIE	Office International des Epizooties (currently WOAH)
PBS	Phosphate Buffer Saline
Pc	Positive Control
PRNT	Plaque Reduction Neutralization Test
RNA	Ribo Nucleic Acid
ROC	Receiver Operating Characteristic
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RVF	Rift valley fever virus
RVV	Rift Valley Virus
UTR	Untranslated Region
WHO	World Health Organization of the United Nations
WNV	West Nile Fever Virus

STATEMENT OF THE AUTHOR

I declare that 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. I solemnly declare that, in this thesis, there is no part which has been submitted to obtain a degree, diploma, or certificate in any institution. It is possible to have a brief excerpt from this thesis without special permission, provided that a truthful acknowledgment of the source is made. Requests for permission for a lengthy quotation from or reproduction of this manuscript in whole or in part may be granted by the principal advisor and head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

Name: _____ Signature: _____

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: _____

DEDICATION

This dissertation thesis is dedicated to my entire family and relatives for their unwavering support and compassion during my academic journey.

List of Published Articles from the PhD Thesis and Study Period

1. Megenas, J. A., Dadi, M. L., Mekonnen, T. K., Larrick, J. W., & Kassa, G. M. (2025). Seroprevalence of West Nile Fever and associated risk factors in livestock of Afar Region, Northeast Ethiopia. *Vet Sci*, 12, 141. https://doi.org/10.3390/vetsci12020141

2. Megenas, J. A., Dadi, M. L., Mekonnen, T. K., Larrick, J. W., & Kassa, G. M. (2024). Seroprevalence of Rift Valley fever and associated risk factors in livestock of Afar Region, Northeastern Ethiopia. *Curr Res Parasitol Vector Borne Dis*, 6, 100215. https://doi.org/10.1016/j.crpvbd.2024.100215

3. Megenas, J. A., Dadi, M. L., Mekonnen, T. K., Larrick, J. W., & Kassa, G. M. (2024). Seroprevalence and co-circulation of Rift Valley fever virus and West Nile fever virus in livestock population of Afar Region, Northeast Ethiopia. *Vet Med Int*, 2024, 8249077. https://doi.org/10.1155/2024/8249077

4. Megenas, J. A., Dadi, M. L., Mekonnen, T. K., Larrick, J. W., & Kassa, G. M. (2024). Knowledge, attitudes, and practices regarding Rift Valley fever and West Nile fever among livestock owners and health professionals in selected districts of the Afar Region, Northeast Ethiopia. *Vet Res Notes*, 4(10), 89–99. https://doi.org/10.5455/vrn.2024.d48

5. Megenas, J.A, Dadi,M.L, & Kassa, G. M. (2022). Seroprevalence and associated risk factors of Rift Valley fever and Crimean-Congo hemorrhagic fever viruses in livestock and their zoonotic potentials: A systematic review. *Veterinaria*. https://doi.org/10.51607/22331360.2022.71.1.1

EPIDEMIOLOGY OF RIFT VALLEY FEVER VIRUS AND WEST NILE VIRUS IN LIVESTOCK POPULATIONS AND THEIR IMPLICATION FOR PUBLIC HEALTH IMPORTANCE IN THE AFAR REGION, NORTHEASTERN ETHIOPIA

Jemberu Alemu Megenas
PhD dissertation
Addis Ababa University, 2025

ABSTRACT

Ethiopia faces heightened risk of Rift Valley fever (RVF) and West Nile virus (WNV) outbreaks due to proximity to endemic countries (Kenya, Somalia, Djibouti), climate-sensitive conditions (heavy rainfall, flooding), climate change, unrestricted livestock movement, and widespread mosquito vectors. Regions like Afar, with tropical ecosystems, are particularly vulnerable, mirroring trends across the Horn of Africa. Despite significant public health and economic threats, the epidemiology of these arboviruses in Ethiopia remains understudied. Therefore, the general objective of the study was to investigate the epidemiological status of Rift Valley Fever and West Nile viruses, along with their associated risk factors, in livestock populations and to assess the public health importance of these diseases in the Afar pastoral region of northeastern Ethiopia. A total of 736 serum samples were collected from 224 cattle, 155 camels, 144 sheep, 121 goats, and 92 donkeys in the Amibara and Haruka districts. The detection of anti-RVF nucleoprotein antibodies of Rift Valley Fever virus and anti- West Nile virus antibodies were performed using the ID Screen® Rift Valley Fever and West Nile competition multispecies ELISA kits. A total of 150 human participants (60 livestock owners, 40 animal health workers and para-veterinarians, and 50 public health professionals) were involved in the KAP study. The overall seroprevalence of RVF was 22.0% (162/736; 95% CI: 19.41–24.79%). The seroprevalence was significantly higher in goats (42.2%, 95% CI: 39.61–44.99%) compared to that of cattle (14.3%, 95% CI: 11.74–17.09%), sheep (21.5%, 95% CI: 18.91–24.29%), or camels (30.97%, 95% CI: 28.38–33.76%) ($P < 0.001$). The study showed that seropositivity for IgG antibody to RVF infection was associated with locality and species of animal. Goats were two times more likely to be seropositive for RVF infection than cattle (OR: 2.3, 95% CI: 1.462–3.574, $P = 0.001$). Livestock in the *Kealatburi* sub-district were five times more likely to be seropositive for RVF infection than those in the *Halidegei* sub-district (OR: 5.074, 95% CI: 3.066–8.396, $P = 0.001$). Among 736 tested livestock serum samples, 50.7% (373/736) showed anti-WNV IgG antibodies evaluated using the ID screen® WNV competition multispecies ELISA kits (95% CI: 47%–54.4%; $P < 0.01$). The seroprevalence was higher ($p<0.01$) in donkeys (76.1%), followed by camels (69.1%), cattle (52.2%), goats (34.7%) and sheep (25.7%), respectively. The study showed a

statistically significant difference of WNV seropositivity between species of animals (OR:1.5, 95%CI =1.038-2.212). Donkeys were (OR: 6.447, 95% CI = 3.888-10.688) seven times more likely to be seropositive for WNV infection than sheep ($p < 0.01$). The study also revealed 9.1%, 95%CI= 8.86-9.29) seroprevalence of co-circulation of RVF and WNV. High 32/155 (20.7%) seroprevalence of co-circulation was seen in camels followed by goat 14/121(11.6%), cattle16/224(7.14%) and sheep 5/144(3.5%) respectively and higher 41/421(9.7%) seroprevalence of co-circulation was observed in Amibara district than Haruka district. Camels were seven times (OR: 7.016, 95% CI = 2.639-18.653) more likely to be seropositive for the co-circulation than sheep ($p =0.000$). Of the participants, 29.3% (44/150) believed zoonotic diseases spread through animal-based food or mosquito bites, while 63.3% (95/150) expressed insecurity about infections like Rift Valley fever, West Nile fever, or other zoonoses. This study found substantial knowledge gaps, a low level of risk concern, and high behavioral practices regarding zoonotic disease. Animal health workers had higher mean scores of knowledges of Rift Valley fever, West Nile fever and other zoonotic disease 27.3 ± 10.9 than public health professionals and livestock farmers/owners at $p< 0.05$. Mean score statistical difference was also observed among Illiterate, primary education, and secondary and above educational status groups. In conclusion, the present study showed that seropositivity of different species of livestock to RVF and WNV is an indication of its widespread occurrence of RVF and WNV in the study area, domestic livestock, especially in large herds, can serve as useful sentinels for the infections and warrants the need for further investigation on molecular epidemiology of RVF and WNV in livestock, their potential zoonotic significance to the pastoral communities and understanding of the vectors in transmission of the viruses in the study area in order to design a feasible control strategy.

Keywords; Afar Region, Epidemiology, KAP, Livestock, Rift Valley fever, Seroprevalence, West Nile Virus, Zoonoses

1. INTRODUCTION

The emergence of novel arboviruses, transmitted by arthropods such as mosquitoes, ticks, and sandflies, has become a significant global threat to public and animal health. These viruses, including Rift Valley fever virus (RVF) and West Nile virus (WNV), are capable of causing a wide spectrum of diseases, ranging from mild febrile illnesses to severe, life-threatening conditions (Salekwa *et al.,* 2019; Holcomb *et al.,* 2023). Arboviruses are maintained in complex transmission cycles involving arthropod vectors and vertebrate hosts, including humans, and their spread is facilitated by factors such as climate change, urbanization, and increased global travel (Fischer *et al.,* 2021; Tinto *et al.,* 2023).

Rift Valley Fever, a zoonotic virus belonging to the *Bunyaviridae* family, primarily affects livestock and humans. It is characterized by a negative-sense single-stranded RNA genome divided into three segments: large (L), medium (M), and small (S). The L segment encodes the RNA polymerase, the M segment contains envelop glycoproteins, and the S segment carries the nucleocapsid protein (Gregor *et al.,* 2021; Bron *et al.,* 2021). RVF causes severe illness in ruminants, including fever, abortions, and high mortality rates in young animals, while humans typically experience mild febrile symptoms, though severe complications such as hemorrhagic fever and encephalitis can occur (Fawzy & Helmy, 2019; Javelle *et al.,* 2020; Wilson *et al.,* 2013).

Since its discovery in Kenya in 1930, RVF has spread across Africa, the Arabian Peninsula, and Indian Ocean islands (Daubney & Hudson, 1931; Alkan *et al.,* 2023). Its epidemiology is complex, involving multiple mosquito species, particularly *Aedes* and *Culex,* which thrive during heavy rainfall episodes (Tong *et al.,* 2019; Fawzy & Helmy, 2019). The virus has significant economic impacts due to livestock losses and trade restrictions, and its potential for geographic expansion raises concerns about future epidemics in new regions (Agboli *et al.,* 2021; Pérez-Ramírez *et al.,* 2020; Hartman*,* 2017).

Humans infected with RVF typically have mild, self-limited febrile illness, but in rare cases (<8%), severe complications such as jaundice, encephalitis, and hemorrhagic

manifestations may occur (FAO, 2009). Retinal degeneration, hemorrhagic fever, or encephalitis may also develop in a small percentage of cases (Nielsen *et al.,* 2020). The primary foci of RVF epidemics are triggered by heavy rainfall, which supports the proliferation of mosquito vectors (Tong *et al.,* 2019).

West Nile Virus (WNV), a member of the *Flaviviridae* family, was first isolated in Uganda in 1937 (Gulati, 2014; Reisen, 2013). It is transmitted primarily by Culex mosquitoes and maintained in a cycle involving birds as amplifying hosts. Humans and horses are incidental hosts, often experiencing mild febrile illness, though severe neurological complications can occur (OIE, 2018). Migratory birds play a key role in the global spread of WNV, which is now endemic in Africa, the Middle East, and parts of Asia (Sayed-Ahmed, 2016; Petersen *et al.,* 2013).

Environmental factors such as heavy rainfall, flooding, and global warming contribute to the proliferation of mosquito vectors, increasing the risk of WNV outbreaks (Yuseri *et al.,* 2019; Cleton *et al.,* 2015). The virus has been documented in numerous African countries, including Ethiopia, where large livestock populations and inadequate veterinary services exacerbate the risk of zoonotic transmission (CSA, 2021; Asebe *et al.,* 2020). WNV also infects domestic animals such as cows, though humans and horses are typically dead-end hosts due to low viremia (Abdelfattah & Abdelhamed, 2020; Kamalia *et al.,* 2022).

Both RVF and WNV are One Health issues, with their transmission dynamics influenced by interactions between humans, animals, and the environment (Dente *et al.,* 2020; Oluwayelu *et al.,* 2018). The presence of competent vectors beyond their current range poses a risk of global spread, particularly as climate change and anthropogenic activities create favorable conditions for vector proliferation (Fischer *et al.,* 2021; Nielsen *et al.,* 2020). Understanding the ecological drivers of these viruses, such as environmental changes and vector behavior, is crucial for predicting and mitigating future outbreaks (Cleton *et al.,* 2015; Tong *et al.,* 2019).

1.1. Statement of the Problem

In Ethiopia, pastoral areas cover approximately 625,000 km², accounting for 57% of the country's total land area, with the Afar Regional State comprising 52% of this pastoral land. The Afar Region, predominantly inhabited by nomadic and semi-nomadic pastoralists, is not traditionally known for settled agriculture. However, medium and large scale commercial irrigated farms have emerged in the Upper and Middle Awash River Basin, one of the most intensively developed basins in the country (Behnke & Kerven, 2011).

The Afar pastoral herds often graze on floodplain vegetation along the Awash River, creating favorable environmental conditions for the proliferation of primary and secondary mosquito vectors, which are critical for the transmission of Rift Valley fever virus (RVF) and West Nile virus (WNV) (Sonneveld *et al.,* 2017). A study by Sang *et al.* (2017) found significantly higher mosquito populations in irrigated farm areas, with rainfall and humidity positively correlated with mosquito densities. Irrigation schemes provide ideal resting and breeding habitats for vectors of RVF and other endemic arboviruses (Tucker *et al.,* 2020).

The Awash River Basin, primarily located in the arid and semi-arid lowlands of the Afar Region, experiences seasonal flooding between August and September due to intense rainfall in the eastern highlands. Tributary rivers draining into the Awash River can rapidly increase water levels, causing flooding in low-lying alluvial plains. Areas such as the marshlands (*Damboo*) north of Awash town near *Melkawerer* are frequently inundated. While these floods improve grazing lands, they also lead to a surge in mosquito populations, particularly after floodwaters recede. Local communities report severe mosquito plagues, with concerns about malaria due to poor health services in the region (Sayed-Ahmed, 2016). A study by Zerfu *et al.* (2018) revealed that 75.4% of participants in the Afar Region had illnesses with unknown causes, highlighting the need for further investigation into febrile illnesses and their causative agents.

Nomadic livestock movements in the Afar Region increase the risk of RVF outbreaks, which can cause significant economic harm to both human and animal health, exacerbating poverty in affected communities (Tigoi *et al.,* 2020). Despite the limited studies on RVF and WNV in Ethiopia, seroprevalence studies indicate the presence of these viruses. For instance, Ibrahim *et al.* (2021) reported RVF seroprevalence rates of 13.2% in humans, 17.9% in cattle, 42.6% in camels, 6.3% in goats, and 7.4% in sheep in the Somali Region. Similarly, Asebe *et al.* (2020) and Endale *et al.* (2021) found RVF seroprevalence rates of 7.6% and 5% in cattle from southwestern Ethiopia and the South Omo area, respectively. Additionally, Endale *et al.* (2021) reported a WNV seroprevalence of 4.8% in cattle from the South Omo area.

Entomological surveys in selected areas of Afar, the mid-Rift Valley, Borena, and Segen Valley identified diverse mosquito species, including *Aedes*, *Culex*, *Anopheles*, and *Mansonia,* which are competent vectors for RVF and WNV (Gutu *et al.,* 2021; Mekuriaw *et al.,* 2022; Jaleta *et al.,* 2022). Although no RVF or WNV cases have been confirmed by reverse transcriptase-PCR in Ethiopia, the country remains vulnerable due to its proximity to endemic regions like Kenya, Djibouti, and Somalia, climate anomalies such as heavy rainfall and flooding, unrestricted livestock movement across borders, and the presence of competent vectors (Jaleta *et al.,* 2022). Despite these risks, no prior studies have investigated the epidemiology of RVF and WNV in the Afar Region, making it a critical area for future research.

To address this gap, a reconnaissance community-based survey and site observation were conducted in June 2021 in the Afar Region. Key observations included a large livestock population, evidence of retained placenta and abortions, the presence of water bodies, extensive irrigation activities, a history of flooding, and proximity to international borders. These factors underscore the region's vulnerability to RVF and WNV outbreaks, necessitating further epidemiological studies to inform prevention and control strategies.

Therefore, the present study was designed with the following Hypotheses:

1.2. Hypotheses

- H_{01}: Rift Valley Fever Virus (RVFV) and West Nile Fever Virus (WNV) are not circulating at significant seroprevalence levels in the livestock population of the Afar Region, Northeastern Ethiopia
- H_{02}: Livestock owners and health professionals in the Afar Region have optimal knowledge, mixed attitudes, and adequate preventive practices regarding RVFV and WNV, contributing to disease persistence or spillover risks.

1.3. Objectives

1.3.1. General Objective

The general objective of this study was to examine the epidemiology of Rift Valley Fever and West Nile viruses in livestock populations and identify the associated risk factors in the Afar pastoral region, specifically within the Amibara and Haruka districts of northeastern Ethiopia. Additionally, the study aimed to assess the public health significance of these diseases and evaluate the knowledge, attitudes, and practices (KAP) of the local communities regarding these zoonotic infections.

1.3.2. Specific objectives

- To determine the seroprevalence of Rift Valley Fever and its associated risk factors in livestock population of two selected districts (Amibara and Haraka) of Afar Region, Northeastern Ethiopia. (Paper- II)
- To investigate the occurrence of West Nile Fever and its associated risk factors in livestock population of two selected districts (Amibara and Haraka) of Afar Region, Northeastern Ethiopia. (Paper- I)
- To determine the seroprevalence and co-circulation of Rift Valley fever virus (RVF) and West Nile fever virus (WNF) in the livestock population of the Afar Region, Northeast Ethiopia, and to identify associated risk factors contributing to their transmission. (Paper -III)
- To assess the community (Livestock owners, health professionals) knowledge, attitude, and perception regarding the public health importance of Rift Valley Fever and West Nile Fever Virus diseases in the study area. (Paper- IV)

2. LITERATURE REVIEW

2.1. Arboviruses

Arboviruses (arthropod-borne viruses) are a diverse group of viruses maintained in transmission cycles between hematophagous arthropod vectors, such as mosquitoes, ticks, midges, and sandflies, and vertebrate animal reservoirs that act as amplifying hosts (Holcomb *et al.,* 2023). Following ingestion via a blood meal from an infected vertebrate, arboviruses replicate initially within the mesenteronal epithelial cells of the arthropod. For many viruses, subsequent dissemination involves secondary amplification in secondary tissues before infecting the salivary glands. However, certain arboviruses can directly infect salivary gland epithelia without requiring this secondary replication phase (Wilson *et al.,* 2020).

Once the salivary glands are infected, the virus is transmitted to a new vertebrate host during subsequent blood feeding. Taxonomically, Arboviruses encompass multiple families, including *Flaviviridae* (*Flavivirus*) and *Bunyaviridae* (*Phlebovirus*), highlighted here (Figure 1).

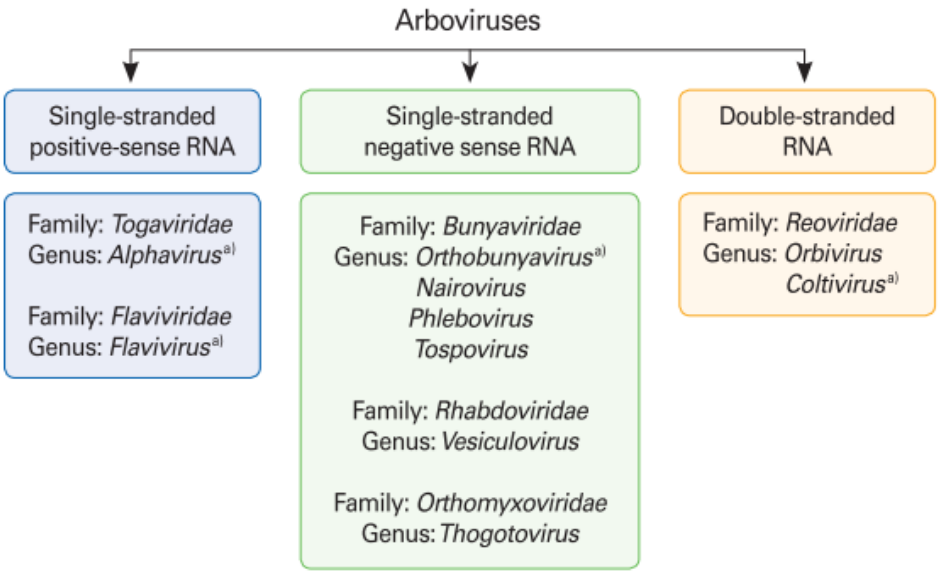


Figure 1: Classification of arboviruses, Source:(Go *et al*., 2014)

2.1.1. Rift Valley Fever Virus (RVF)

2.1.1.1. The Organism

One of the most significant members of the vast and varied Bunyaviridae/Phenuiviridae family is the Rift Valley fever virus (RVF). The Bunyaviridae are a very large family of single-strand, enveloped RNA viruses (more than 300 viruses) and consists of five genera ofviruses: Orthobunyavirus, Phlebovirus, Nairovirus, Hantavirus,and Tospovirus (Tospov iruses infect only plants)(Pawęska *et al.,* 2021).

The name *phlebovirus* is derived from the fact that most of the viruses in this genus are transmitted by phlebotomine sandflies(Ikegami & Makino, 2011). RVF is a notable exception from the group because it is transmitted primarily by mosquitoes. All *bunyaviruses*, including RVF, contain a tripartite genome consisting of 3 single-stranded, negative polarity RNA segments. The genome segments are creatively named large (L), medium (M), and small (S). The L segment encodes the viral polymerase protein. The M segment encodes the glycoproteins (Gn and Gc), as well as a non-structural protein termed NSm. Both L and M segments use a negative-sense coding strategy(Sado *et al*., 2022).

In contrast, *phleboviruses* use an ambisense coding strategy for the S segment. The nucleoprotein (N) is encoded with negative polarity on the S segment, while a second non-structural protein, NSs, is encoded with positive polarity. Nucleocapsids consist of the viral nucleoprotein (N) multiplexed with each RNA segment. Complementary nucleotide sequences at the 3′ and 5′ ends of each segment are thought to form circular RNAs(Sharma, 2009).

Cells become infected with RVF by receptor-mediated endocytosis, followed by pH mediated fusion of virus-endosomal membranes to release nucleocapsids into the cell cytoplasm. Transcription, translation, and genome replication occur in the cytoplasm. A unique aspect to the life cycle of RVF and other bunyaviruses is that mature viral particles assemble and bud from the Golgi apparatus in some cell types. Genomic sequence analysis

of strains obtained between 1944-2007 reveal relatively low (5%) sequence divergences. There are at least seven genetic lineages, yet lineage does not correlate with geographic origin indicating that there is substantial regional movement of genotypes. Genetic evidence exists for the reassortment of viral segments in nature but no indication of recombination(OIE, 2018).

2.1.1.2. History

An outbreak of "an obscure disease that caused heavy mortality in lambs" was reported more than a century ago in June 1912, and it momentarily had a depressing effect on the sheep industry in Kenya (Table 1). Rift Valley fever virus (RVF), the most likely causative agent, was isolated by Daubney and associates in 1930, eighteen years later. Nearly a century has passed since the Rift Valley fever *phlebovirus*, which is mainly transmitted by mosquitoes, was discovered. It continues to infect animals and cause deaths, spreading to humans in the Middle East, Africa, and the Indian Ocean. Many times, impoverished communities' livelihoods can be upended by outbreaks, which can result in significant, recurrent financial losses. People and animal populations are at risk from RVF because of its economic impact, pathogenicity, and unpredictable (re)emergence(Bron *et al*., 2021).

Daubney, Hudson, and Garnham, along with other employees at the Veterinary Research Laboratory at Kabete, Kenya, first described Rift Valley fever (RVF) in 1934. Stordy, who worked in the same department in 1913, had previously reported on a disease syndrome that resembled RVF. This disease showed up in the Rift Valley in exotic wool sheep that had been brought into East Africa and was acute and highly fatal(Daubney & Hudson, 1931; Davies, 2010).

An association of the disease with heavy and prolonged rainy seasons was noted. Epizootics occurred periodically in Kenya until the disease was recognized in South Africa in 1951, when humans became ill after handling dead and infected animals. Sheep and to a lesser extent cattle were the principal disease hosts in both east and southern Africa(Johnson *et al*., 2012). Further epizootics were subsequently confirmed in

Zimbabwe(Ndengu *et al*., 2020), Zambia(Braack *et al*., 2018), the Sudan(Seufi & Galal, 2010), and other east African countries(Daubney & Hudson, 1931).

In 1977 there was a major epidemic in Egypt, with 20–40,000 clinical illnesses and 600 deaths(Zouaghi *et al*., 2021). Cattle and sheep suffered from abortions and neonatal mortality; goats, camels, and water buffalo were also affected. Subsequently, RVF was identified in West Africa in Senegal and Mauritania, where human mortality was again high(Braack *et al*., 2018). In 2000, an outbreak occurred in Saudi Arabia, the first occurrence of RVF virus outside Africa(Zouaghi *et al*., 2021). The ecology there is identical with that in enzootic zones in Africa and the RVF, which circulated were the same biotopes as were seen in Africa. Today, it is generally acknowledged that RVF is enzootic throughout the African continent and Saudi Arabia, and in many African countries, although disease has not been recognized in man nor in animals in a substantial proportion of enzootic countries(Leta *et al*., 2018).

Table 1: Historical data on the outbreak of Rift Valley Fever

Years	Outbreak Happening Country
1910-12	Disease compatible with RVF described in lambs (European breed) in rift valley, Kenya
1930	Virus first isolated in outbreak of sheep disease in rift valley, Kenya
1930-44	Subsequent recognition of presence of virus in many sub- Saharan countries
1944	Isolation of RVF virus in semliki forest Uganda (no livestock or humans in vicinity) – hence RVF assumed to be endemic in forests with spread to grasslands after heavy rains
1950-1	Large outbreak in South Africa – associated with pans & vleis (dambos) - ocular lesions recognized
1976	Large outbreak in South Africa – fatal human disease recognized for first time
1977-8	Appearance of RVF beyond sub-Saharan Africa -in Egypt - >200,000 human infections – 598 deaths
1979	Recognition of RVF in Madagascar
1987	Large outbreaks in Mauritania/Senegal – many human deaths
1997-8	Large outbreak in Kenya/Somalia/Tanzania - >300 human deaths
2000-1	Appearance of RVF beyond African region in Saudi Arabia & Yemen – >200 deaths
2006-7	Large outbreak in Kenya/Somalia/Tanzania

2007	Outbreak in Sudan
2008	Madagascar: The Ministry of Health, Madagascar reported an outbreak of RVF on 17 April 2008. From January to June 2008, a total of 476 suspected cases of RVF including 19 deaths were reported from 4 provinces.
2008–2009	Madagascar: From December 2008 to May 2009, the Ministry of Health, Madagascar reported 236 suspected cases including 7 deaths.
2010	Republic of South Africa: From February to July 2010, the Government of South Africa reported 237 confirmed cases of RVF in humans, including 26 deaths from 9 provinces.
2012	Republic of Mauritania: The Ministry of Health in Mauritania declared an outbreak of RVF on 4 October 2012. From 16 September 2012 (the date of onset of the index case) to 13 November 2012, a total of 36 cases, including 18 deaths were reported from 6 regions.
2016	Republic of Niger: As of 11 October 2016, Ministry of Health reported 105 suspected cases including 28 deaths of RVF in humans in Tahoua region.
2020	Mauritania: The Ministry of Health (MoH) notified WHO that between 13 September and 1 October 2020, eight cases of Rift Valley Fever (RVF) including seven deaths were confirmed in animal breeders.
2021	Rift Valley fever (RVF) has been reported in Kenya in humans in Isiolo and Mandera counties and in animals in Isiolo, Mandera, Murang'a and Garissa counties. As of 4 February 2021, there were a total of 32 human cases (14 confirmed positive), and 11 deaths (CFR 34 %).
2022	a total of 47 confirmed cases including 23 deaths (CFR 49%)—mostly among animal breeders— have been reported from nine of Mauritania's 15 wilayas. Among the 47 confirmed cases, there are more men than women.

Source;(Paweska, 2008; WHO, 2023)

2.1.1.3. Epidemiology

The epidemiology of RVF is complex and involves multiple players, including mosquitoes, wild animals, domesticated livestock, and humans(Hassan *et al*., 2020).

2.1.1.4.1. Geographical Distribution

Rift Valley fever virus has a broad geographic distribution across Africa (Figure 2) with enzootic and epizootic cases reported throughout much of the continent, specially endemic in Africa south of the Sahara Desert(Alomar *et al*., 2023).Although outbreaks are most

common in southern and eastern Africa, they also occur in other regions. Outbreaks or infections have been reported sporadically in Egypt and various islands off the coast of Africa (e.g., Madagascar, Mayotte), and a major outbreak occurred on the Arabian Peninsula (Saudi Arabia and Yemen) in 2000-2001(Johnson *et al*., 2012). Rift Valley fever might have become endemic in some of these regions, including Egypt; however, this can be difficult to determine, both because the virus is not necessarily found during interepidemic periods, and because some reported infections may result from illegal animal importation(Oluwayelu *et al*., 2018).

However, the frequency and distribution of reported outbreaks have increased, particularly since 2000, when the virus spread to the Arabian Peninsula. Multiple epizootics among domestic animals occurred between 2000 and 2020 (Figure 1), including in Senegal, Mauritania, and the Gambia in West Africa between 2012 and 2016; in South Africa and Namibia beginning in 2009; and in Angola in 2016. Niger, a country that is part of the Sahel and located in northern Central Africa, also had a moderate RVF epizootic in 2016 In 2006 and 2007, a large epizootic occurred in Kenya, Somalia, and Tanzania in East Africa, following heavy rains associated with El Niño Southern Oscillation (ENSO) climatic conditions(Lichoti *et al*., 2014; Taylor *et al*., 2016).

In 2018, another epizootic occurred in Kenya, also following unusually heavy rains, this time during the long rainy season with human cases confirmed in June(Mor *et al*.). In 2016, a multi-year epizootic began in Uganda and was the first laboratory-confirmed detection in this country in 48 years(Shoemaker *et al*., 2019). In 2019, multiple human and animal cases were detected on the Island of Mayotte off the coast of East Africa in the Indian Ocean(Tinto *et al*., 2023). The large number of mosquitoes capable of transmitting RVF, the diversity of susceptible vertebrate hosts, and the distribution of the virus across a broad range of bioclimatic conditions make understanding its potential establishment and spread into new geographic areas a global priority (Juma *et al*., 2022; Pepin *et al*., 2010).

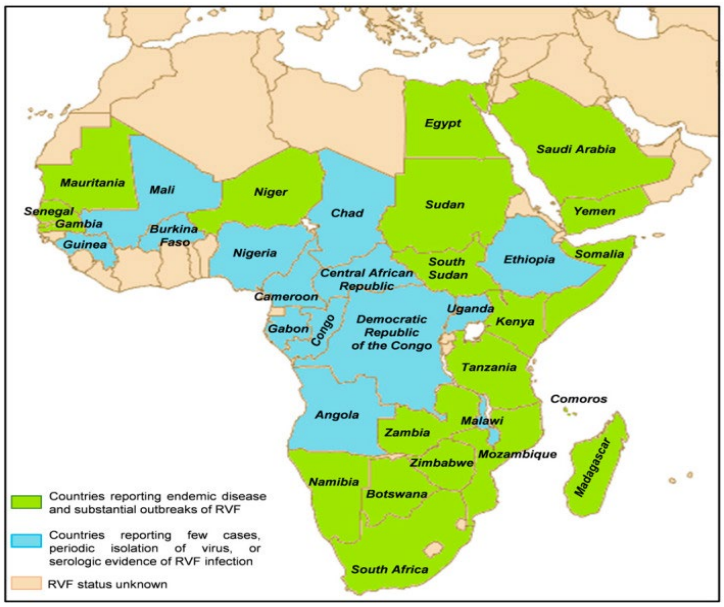


Figure 2: RVF distribution map.

Source;(Lapa *et al*., 2024)

The map delineates Rift Valley Fever (RVF) endemicity across Africa and the Middle East, categorizing countries as follows: ***endemic zones with substantial outbreaks*** *include Senegal ("Somegal"), Gambia, Guinea, Mali, Burkina Faso (erroneously split as "Burkina" and "Faso"), Niger, Nigeria, Chad, Central African Republic, South Sudan, Uganda, Yemen, Somalia, Cameroon, Gabon, Democratic Republic of the Congo, Kenya, Tanzania, Angola, Zambia, Malawi, Mozambique, Zimbabwe, Botswana, and Namibia;* ***South Africa*** *is listed separately, suggesting distinct epidemiological patterns. Regions with* ***few cases or serologic evidence*** *and* ***unknown RVF status*** *are implied but not explicitly detailed. The categorization underscores RVF's pervasive presence in sub-Saharan Africa, particularly in flood-prone and pastoral ecosystems, emphasizing the need for targeted surveillance and mitigation strategies in high-risk zones.*

Establishment in new geographic areas could occur through the introduction of viremic ruminants, including domestic livestock, which could then infect competent mosquito vectors in the new region. This scenario has been hypothesized as the means of introduction from Africa to the Arabian Peninsula (Tigoi *et al*., 2020). Other potential routes of establishment have been deemed to be relatively low risk but not impossible, including the

movement of infected mosquito vectors into a new geographic area (Figure 3) through trade or travel.

There is also limited evidence that infected humans traveling to a non-endemic location could play a role in RVF emergence by serving as an infectious reservoir and triggering an outbreak(Golnar *et al*., 2014). However, although RVF amplification in humans to mosquito-transmissible levels has been documented, in areas where RVF is established, human hosts likely play a negligible role in transmission cycles compared to other mammalian hosts. Nevertheless, the travel of infectious humans should not be ignored as a potential route of RVF introduction and emergence. The risk of establishment through intentional introduction from a bioweapon remains unknown but it is certainly a concern for the United States and other non-endemic countries with conditions conducive to a sustained transmission cycle.

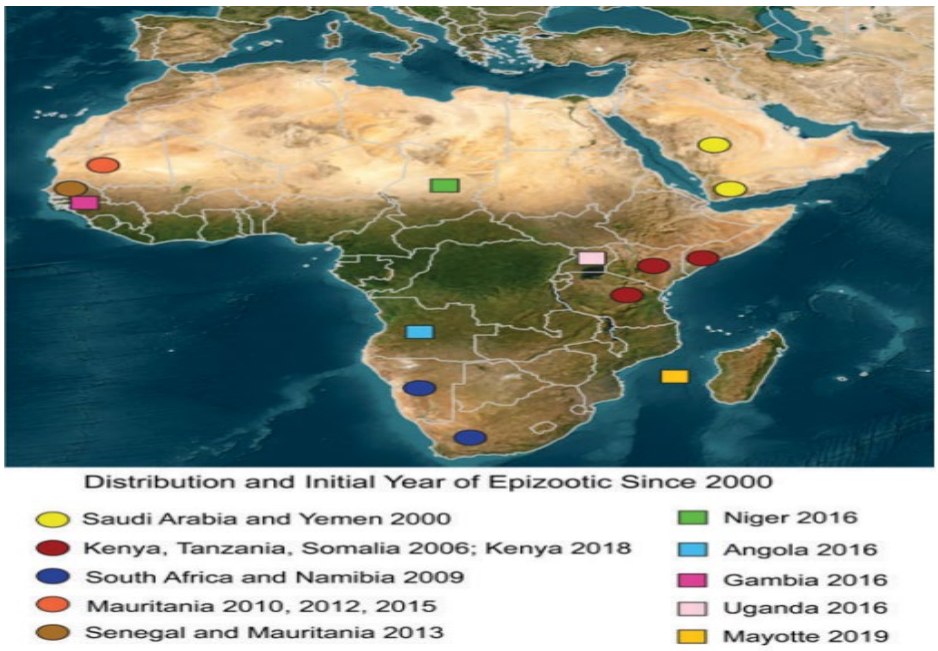


Figure 3: RVF epizootics between 2000–2020

Source;(Alomar *et al*., 2023)

The map illustrates the geographical distribution and initial years of epizootic (animal disease outbreak) events reported since 2000.

1. Early Outbreaks (2000–2006):
    - 2000: The earliest recorded epizootic occurred in Saudi Arabia and Yemen.
    - 2006: A multi-country outbreak affected Kenya, Tanzania, and Somalia. Kenya later experienced another outbreak in 2018.
2. Southern Africa (2009):
    - 2009: South Africa and Namibia reported outbreaks.
3. Recurring Outbreaks in Mauritania (2010–2015):
    - 2010, 2012, 2015: Mauritania faced repeated outbreaks.
    - 2013: The outbreak expanded to include neighboring Senegal.
4. Widespread Outbreaks in 2016:
    - 2016: A significant year with outbreaks in Niger, Angola, Gambia, and Uganda, indicating a broad geographic spread across Africa.
5. Recent Activity (2019):
    - 2019: The island of Mayotte (French territory near Madagascar) reported an outbreak.

Patterns:

- Regional Clusters: Outbreaks often occurred in neighboring countries (e.g., Mauritania and Senegal; Kenya, Tanzania, and Somalia).
- Recurrence: Mauritania and Kenya experienced repeated outbreaks over multiple years.
- Temporal Spread: The 2016 outbreaks highlight a year of heightened activity across diverse regions.

2.1.1.1.2. Rift Valley Fever Virus Vectors and Vertebrate Hosts

2.1.1.4.1.1. Mosquitoes

Various mosquito species from at least six genera have been discovered in different parts of Africa that are naturally infected with the virus and present during RVF outbreaks(Linthicum *et al*., 2016). The majority of RVF isolations from mosquitoes have come from female members of the genus Aedes, which is rich in species and biologically diverse, with about 1,270 species and 75 subgenera worldwide(Wilkerson *et al*., 2015).

Approximately three quarters of African Aedes species from which RVF has been isolated are considered floodwater mosquitoes, a biologically convergent group that lays eggs in shallow ground depressions that will become pools of water after heavy rainfall or flooding. Mosquitoes collectively referred to as floodwater *Aedes* have been classified as the primary vectors of RVF, maintaining the virus through trans-ovarially infected drought, resistant eggs that survive in dry soils on low lying depressions on land over inter-epidemic periods that could be as long as 5 to 15 years(Rosemary Sang *et al*., 2017). Mosquitoes are both a reservoir and vector for RVF, which means that they can maintain viruses for life and transmit it to their offspring via eggs. After periods of heavy rainfall and flooding, an increased number of RVF infected mosquitos may hatch and pass viruses to people and animals.

RVF vectors can be classified into two major groups, namely primary and secondary vectors(Poueme Namegni *et al*., 2020). The primary vectors, according to the literature, are mosquitoes of the genus Aedes that maintain RVF by trans-ovarially transmitting the virus to the next generation. They usually lay these infected eggs in geomorphic structures in the form of shallow depressions without a stream, which can be covered with grass and are called dambos in East Africa. The eggs of these primary vectors are resistant to desiccation and can therefore diapause in dry depressions for long periods of time, and during periods of high precipitation, infectious mosquitoes can hatch. This can cause the virus to be transmitted to nearby animals and humans when the vectors search for blood meals. Once primary transmission of the virus has taken place, secondary vectors

belonging to other genera, such as *Culex*, *Anopheles*, and *Mansonia*, which invade flooded grounds for breeding, contribute to the amplification of the virus, consequently resulting in outbreaks due to their ubiquitous biting patterns. Following the outbreak of RVF in Mauritania in 1987, entomological and veterinary studies (Table 2), initiated in Senegal to contribute to the understanding of the epidemiology of this arbovirus, led to isolation of the virus in different mosquito species (such as *Aedes vexans* and *Ae. ochraceus*), suggesting a possible enzootic cycle of RVF transmission in West Africa. Additionally, this virus has been isolated from *Culex poicilipes*, *Ae. vexans*, *Mansonia africana*, and *Ae. fowleri* in Barkedji. In Kenya, the primary vectors *Ae. mcintoshi* and *Ae. ochraceus* have been reported to serve as reservoirs of the virus(Terasaki *et al*., 2016).

Rift Valley Fever Virus has also been isolated from other mosquito genera (Table 2), as well as ticks, flies, and midges, but their role in biological transmission is unknown(Linthicum *et al*., 2016). A unique aspect to the biology of RVF is that the virus is maintained by transovarial transmission within Aedes mosquito eggs, meaning that live virus can be passed from parent mosquito to offspring by maintenance within eggs(Sang *et al*., 2010). During inter-epidemic periods, the virus remains infectious within dormant desiccated Aedes mosquito eggs in dry floodplains; infected mosquitoes will emerge during flooding. As would be expected, outbreaks are associated with unusually heavy rainfall, especially cyclical El Nino-Southern Oscillation (ENSO) weather patterns(Lancelot *et al*., 2017).

Table 2: References from which information obtained regarding vector potentially transmitting RVF

Mosquito species	Indoor	Near water	Outdoor	Number and per cent	References†
Ae. mcintoshi	-	5	-	5 (0.3%)	Pepin et al. (2010)
Ae. ochraceus	-	116	10	126 (8.0%)	Pepin et al. (2010)
Ae. vexans	4	17	10	31 (2.0%)	Tantely et al. (2015)
Ae. bromeliae	-	8	-	8 (0.5%)	
Ae. cumminsii	-	4	-	4 (3.0%)	Tantely et al. (2015)
Ae. geniculatus	-	48	12	60 (3.9%)	
Ae. furcifer	6	11	2	19 (1.2%)	
Other *Aedes species*	4	105	34	143(9.7%)	
An. arabiensis	8	37	9	54 (4.0%)	Tantely et al. (2015)
An. gambiae	5	56	3	64 (2.9%)	Tandina et al. (2018)
Other *Anopheles species*	47	299	40	386 (24.5%)	
Cx. antennatus	70	26	24	120 (7.6%)	Linthicum et al. (1985), Pepin et al. (2010), Tandina et al. (2018)
Cx. univitattus	3	97	5	105 (7.0%)	Tandina et al. (2018)
Cx.quinquefasciatus	13	124	5	142 (9.0%)	Tandina et al. (2018)
Cx. pipiens	34	19	11	64 (4.1%)	Pepin et al. (2010)
Cx. theileri	15	14	6	35 (2.2%)	Pepin et al. (2010)
Mansonia uniformis	56	113	41	210 (13.3%)	Tantely et al. (2015)
Total	265	1099	212	1576	
%	16.8%	69.7%	13.5%	100.0%	

Source;(Jaleta *et al*., 2022)

2.1.1.4.1.2. Mammals

Rift Valley fever can affect many species of animals (Table 3) including sheep, cattle, goats, African buffalo (*Syncerus caffer*), water buffalo (*Bubalus bubalis*), camels, some species of monkeys, and several rodents (various wild African rodents, rats [*Rattus rattus*], nonnative gray squirrels [*Sciurus carolinensis*] and laboratory rodents including hamsters, gerbils, rats, and mice, but not guinea pigs). Severe disease can be seen in newborn puppies and kittens, although adult dogs and cats seem to be unaffected. Some experimentally infected ferrets developed febrile reactions. Limited information suggests that some wild ruminants (in addition to African buffalo) are also susceptible to disease (Roger *et al*., 2014).

Sheep, goats, and cattle are thought to be the primary amplifying hosts among domesticated animals, although other species such as camels could also be involved (Oluwayelu *et al*., 2018). The role of wildlife is still being investigated, but some animals might amplify the virus or help maintain it during interepidemic periods. In addition to African buffalo, serological evidence of infection has been reported in other wild ruminants (e.g., Thomson's gazelle [*Gazella thomsonii*], lesser kudu [*Tragelaphus strepsiceros*], impala [*Aepyceros melampus*] and waterbuck [*Kobus ellipsiprymnus*]), as well as in black rhinocerus (*Diceros bicornis*), African elephant (*Loxodonta africana*) and warthog *(Phacochoerus aethiopicus*) (Oluwayelu *et al*., 2018).

Some wild species, such as giraffes, seem to become infected mainly during outbreaks in domesticated animals, and may be unlikely to maintain the virus. Wild rodents have been proposed as possible hosts for RVF virus, but their role (if any) is currently uncertain. There is also evidence of infection in other species including bats. Birds do not become infected in laboratory experiments, and surveillance has not detected any evidence of infection in nature(Sadeuh-Mba *et al*., 2018).

The incubation period in sheep, goats and cattle is thought to be approximately 1-3 days, based on laboratory experiments. Young ruminants and puppies can develop clinical signs as early as 12 hours after inoculation. Like most arboviruses, RVF alternates between mosquitoes and vertebrate hosts. Evidence of RVF infection (as determined by hemagglutinin inhibition or plaque-reducing neutralizing antibody titers) has been found in many wild mammalian species in Africa, including bats, lions, and elephants(Metras *et al*., 2020).

The virus causes mild or apparent illness in these species. It is not known whether any of the wild animal species are amplifying hosts. Unlike wild animals, RVF is highly pathogenic in domesticated ruminants, which are the amplifying hosts, meaning they develop sufficient viremia to infect feeding mosquitoes and potentiate further transmission. The most severe disease occurs in developing fetuses and very young animals immediately

after birth; older animals are somewhat more resistant. High titers of virus are found in the blood of infected animals for approximately a week after onset of illness. Direct animal-to-animal transmission of RVF does not occur among herds or experimentally in the laboratory. Culex and Mansonia mosquitoes are thought to be responsible for horizontal transmission between viremic animals and humans(Heinrich *et al*., 2012).

Table 3: Wild and domesticated animals with evidence of RVF infection

Domesticated animals	Wild animals	
Cattle	Springbok	Elephants
Sheep	Wildebeest	Bats
Goat	Impala	Rhinoceros
Buffalo	Lions	Murine rodents
Camel	Gazelles	
Horses	African buffalo	
Donkeys	Warthogs	

Source:(Hartman, 2017)

2.1.1.2. Clinical appearance of Rift Valley Fever in Animals

RVF can affect many species of animals. Sheep and cattle are most severely affected, and they are the primary amplifying hosts for the virus(Terasaki *et al*., 2016). Adult sheep and cattle may develop clinical disease and abortions can reach 100%. In kids and calves' clinical disease is severe and mortality is high. Other ruminants are also affected (Table 4). Generally, adults do not show clinical disease, but abortion and disease of young animals can be severe. The same is true for dogs and cats. Some rodents are susceptible, and others are resistant. Neutralizing antibodies has been reported in a small percentage of horses and some monkeys in areas where the disease is endemic. Pigs, birds, rabbits, guinea pigs, and others appear to be resistant to the virus. Abortion in adult sheep and goats is the most common sign of RVF. It can occur at any state of gestation. The fetus will have an

autolyzed appearance. Abortion rates are very high in some cases as high as 100%(Van den Bergh *et al*., 2019).

Adult sheep can have inapparent infections. Clinical signs most seen include fever, mucopurulent nasal discharge and possibly vomiting. Mortality in adults, especially those that have aborted, can be 20 to 30%; however, abortion may be the only sign seen. The incubation period in lambs and kids is 12 to 36 hours. As previously mentioned, aborted fetuses are the most common sign. Newborns are highly susceptible. Signs include high fever (105.8 ºF), listlessness, and anorexia. Most lambs die within 2 days but can occur in as short as 12 hours. Mortality can be over 90% for young animals less than 1 week old. Lambs and kids over 2 weeks old have a mortality rate over 20%(Fafetine *et al*., 2013).

Cattle are also affected by RVF. Adults usually have inapparent diseases. Clinical signs seen include fever, weakness, anorexia, excessive salivation, and fetid diarrhea. Icterus is also commonly seen. Death in adult cattle can be 10%. Abortions also occur in cattle and can be as high as 100%. Calves show similar signs as lambs and kids - fever, depression, and acute death. Mortality in calves can be from 10 to 70%(Matiko *et al*., 2018).

Other species can be infected by RVF, but such cases are less common. Dogs can have abortion rates as high as 100%. Puppies are severely affected and typically die. Kittens have also been reported to be highly susceptible to RVF virus. Horses have been experimentally shown to have a low-grade viremia; however, to date there have been no equine cases. Pigs have been reported to either be very resistant to the virus or have inapparent infections. Birds have been found to be refractory to the virus(Mayer *et al*., 2017).

Table 4: Rift Valley Fever disease in domesticated animals

	Adult Sheep or Goats	Lambs or Kids	Adult Cattle	Calves
Per acute disease?	yes	no	yes	no
Acute clinical disease	fever, weakness, anorexia, diarrhea, bloody nasal discharge, jaundice, increased respiration; leukopenia, elevated liver enzymes, jaundice	high fever, listlessness, inactivity, anorexia; abdominal pain; increased respiration rate; bleeding and hemorrhage from the ruminant stomach; blood in intestine	weakness, anorexia, bloody diarrhea, hypersalivation, blanore	jaundice, fever, weakness, anorexia, bloody diarrhea
Macroscopic pathology	Liver appears mottled due to cell necrosis and hemorrhage; enlarged lymph nodes.	enlarged, friable liver (mottled due to necrotic foci and hemorrhage); spleen: capsular hemorrhage with some enlargement; enlarged lymph nodes.	Liver appears mottled due to cell necrosis and hemorrhage; enlarged lymph nodes.	Enlarged, friable liver; enlarged spleen with some hemorrhage; enlarged lymph nodes.
Microscopic pathology	hepatic and splenic necrosis (less severe and extensive than lambs)	hepatic necrosis; lung congestion; hemorrhage in mucosa of abomasum; necrosis of spleen; pyknotic/karyorrhexic kidney; sporadic necrosis in small intestine	hepatic and splenic necrosis (less severe and extensive than calves)	hepatic and splenic necrosis; lung congestion
Time frame	3 days	before 36 hours	2–3 days	2–8 days
Mortality rate	30-50%	90%	5–10%	20%
Fetal effects	frequent abortion (up to 100%)		Comparatively less frequent abortion (up to 85%)	

Source;(Hartman, 2017)

2.1.1.2. Human

In humans, the incubation period is estimated to be 3 to 6 days. This is based on a limited number of cases, almost all acquired in the laboratory or by contact with the tissues of infected animals, rather than after exposure to mosquitoes(Nyakarahuka *et al*., 2018).

Most people are infected sub clinically with RVF virus or develop a mild to moderate, non-fatal, febrile flu-like illness with liver abnormalities. The symptoms (Table 5) of uncomplicated infections are usually nonspecific and may include fever, headache, generalized weakness, dizziness, weight loss, myalgia and back pain. Some patients also have stiffness of the neck, photophobia and vomiting or diarrhea. Most people recover spontaneously within a week. A clinical syndrome that appears to be characteristic of severe Rift Valley fever was described in several patients during the 2006-2007 epidemic in Kenya, and included nonspecific signs of fever, malaise, and headaches, together with arthralgia in the large joints (elbows, knees, and shoulders), gastrointestinal signs (nausea, vomiting, mid epigastric pain), progressing to tender hepatomegaly, jaundice and delirium. Lymphadenopathy and diarrhea appeared to be absent in these patients(Metras *et al*., 2020).

Complications including renal dysfunction, meningoencephalitis, ocular disease, or hemorrhagic syndrome with liver involvement occur in a small percentage of patients. The hemorrhagic syndrome is the most serious form and may be seen in up to 1% of patients, with symptoms usually developing 2-4 days after the initial signs. Jaundice may be the first indication of this syndrome, followed by signs such as hematemesis, melena, menorrhagia, a macular or purpuric rash, petechiae and bleeding from the gums. These patients often progress to frank hemorrhages, shock, and death within 3-6 days. Acute kidney dysfunction is a new complication reported in severe cases during recent outbreaks and can also lead to death. Whether the kidney dysfunction is caused directly by the virus or is a consequence of complications such as shock is still unclear. Survivors of this syndrome did not develop chronic kidney disease. Ocular disease and meningoencephalitis can be late complications of Rift Valley fever(Kwasnik *et al*., 2021).

The ocular form is estimated to occur in up to 2% of patients, typically begins 1-3 weeks after the initial symptoms, and is characterized by retinal lesions and blurred vision. While ocular lesions disappear after 10-12 weeks in some patients, others experience some degree of permanent visual impairment, which may include blindness. Encephalitic signs are seen in less than 1%, and usually begin 1-4 weeks (but occasionally later) after the initial signs. The symptoms can include intense headache, memory loss, vertigo, hallucinations, confusion, disorientation, coma or seizures. Some patients have permanent neurological damage, which may be severe, but deaths are uncommon. Some authors indicate that increased mortality or abortions in pregnant women have not been seen during outbreaks. However, there are at least two reports of maternal transmission to the fetus. One infant, whose mother was also ill with malaria, was borne with a rash and jaundice. The infant in the other case died soon after birth(Kwasnik *et al*., 2021).

In the animals, transmission is mainly through bites of Aedes mosquitoes. However, the disease is mainly acquired in humans through contact with blood, body fluids, or tissue and consumption of raw or undercooked milk or meat from infected animals. This is why people who interact with animals and their products such as veterinarians, herders, and butchers are a high-risk population for RVF(Bronsvoort *et al*., 2022).

The disease has an incubation period of 2–6 days in humans and varies in severity; that is, some remain asymptomatic and others might experience mild illness whereas some may have severe disease(Bronsvoort *et al*., 2022). Rift Valley fever virus patients may present with fever, generalized weakness, back pain, and dizziness which can clear within 2–7 days. However, 8–10% can develop severe disease characterized with ocular disease, encephalitis, or haemorrhagic fever. About 50% of those that develop severe disease either die or remain with permanent disabilities(Sadeuh-Mba *et al*., 2018). RVF outbreaks have mainly been reported in Sub-Saharan Africa; for example, in 2006 through 2007, RVF occurred in East Africa affecting more than 1,000 people with 300 deaths(Bronsvoort *et al*., 2022). Uganda reported an RVF outbreak among humans in 2016 and since then over ten sporadic RVF outbreaks have occurred(Kemunto *et al*., 2018).

Humans become infected with RVF when there is widespread illness and death among domesticated livestock. People can be infected by the bite of a mosquito, but the primary means of transmission of the virus to people is thought to be through mucous membrane exposure or inhalation of viral particles during the handling of infected animals and carcasses(Ebogo-Belobo *et al*., 2023).

A number of retrospective studies suggest that touching/handling, living close to, and consuming animal products are factors associated with increased likelihood of RVF infection and possibly more severe outcomes(Hassan *et al*., 2020). Between 1997–2010, there were 9 RVF outbreaks, with 1,220 confirmed human deaths and >500,000 estimated human cases(Budasha *et al*., 2018). Most recently in March of 2016, human cases of RVF, associated with an outbreak in goats, occurred in Uganda. One of these patients was a butcher, and the other reported to have interacted with sick animals. The virus has not shown direct human-to-human transmission, and there have been only a few documented cases of vertical transmission(Birungi *et al*., 2021).

Table 5: Human clinical symptoms of Rift Valley Fever

Uncomplicated	Ocular complications	Hemorrhagic complications	Meningoencephalitis
Headache	Vision reduction	Jaundice	Severe headache
Body aches	Blind spots	Blood in urine/feces	Hallucination
Fever	Photophobia	Vomiting blood	Disorientation
Abdominal pain	Retro-orbital pain	Purple rash	Vertigo
Joint/muscle aches	Uveitis	Gingival bleeding	Excessive salivation
Vomiting	Retinitis	Weakness	
Anorexia	Partial paralysis		
Weakness			
Nosebleeds			
Sweating			
Constipation			

Source;(Javelle *et al*., 2020)

2.1.1.3. Transmission

Rainfall and vector ecology play a major role in the transmission cycles (Figure 4) of RVF because the primary vectors, floodwater Aedes species, need to hatch their eggs for the virus to spread. Biological carriers, mosquitoes, are the primary means of transmission of Rift Valley fever(Alomar *et al*., 2023). This virus has been detected in many genera of mosquitoes in endemic regions; however, laboratory experiments suggest that some of these species are not competent vectors for transmission. At present, the major hosts appear to be members of the genera *Aedes, Culex* and *Anopheles*(Olive *et al*., 2016).

The mechanisms that maintain RVF virus in nature and cause it to emerge in epidemic form are incompletely understood and might differ between areas. Transmission cycles are best understood in savannah regions, where the virus is thought to survive between outbreaks in the dried eggs of *Aedes* mosquitoes found in shallow depressions in the soil (*dambos*). Infected mosquitoes are thought to hatch when the *dambos* fill after heavy rainfall, and initiate transmission cycles involving additional mosquito species, and animals that act as amplifying hosts. The vertebrate amplifying hosts are thought to be critical in propagating epidemics. Virus transmission has also been demonstrated at low levels in livestock, wildlife, and humans during interepidemic periods. Infection cycles in some other climates, such as forested regions, are poorly understood and might differ from this pattern(Sang *et al*., 2017).

RVF virus can be transmitted in utero to the fetus of ruminants, camels, and other species. This virus may also infect other animals exposed to abortion or birth products, which contain large amounts of virus; however, the importance of this route in propagating epizootics is controversial. Although RVF virus can enter the body through mucous membranes, and might occur in milk, one attempt to inoculate puppies, kittens and lambs with virus spiked milk was unsuccessful. Virus shedding in secretions and excretions from infected ruminants is poorly understood, although it is not thought to be important in spreading Rift Valley fever. Some studies have, nevertheless, detected small amounts of virus in oral fluids and nasal discharge, as well as in semen, and sentinel sheep were

infected by unknown route(s) during two laboratory experiments. There was evidence for horizontal transmission during experiments in cats and dogs, and virus was detected in the saliva of puppies(Shoemaker *et al*., 2019).

RVF virus has not been reported in the urine or feces of any species except when these excretions are contaminated by blood. Humans can acquire RVF virus by direct contact with infected tissues, contact with aerosolized viruses generated in laboratories or during slaughter, or from mosquitoes. Drinking raw (unpasteurized) milk is a significant risk factor for human infection, although definitive proof for this route is lacking. Vertical transmission to human infants has been demonstrated in at least two cases(Golnar *et al*., 2014).

In vitro experiments suggest that RVF virus can persist for a few days in some protein-rich environments such as tissues. In a neutral or alkaline pH, mixed with serum or other proteins, the virus may survive for as long as 4 months at 4°C (40°F) and 8 years below 0°C (32°F). It is quickly destroyed by pH changes in decomposing carcasses. Under optimal conditions, RVF virus remained viable in aerosols for more than an hour at 25°C (77°F)(Kwasnik *et al*., 2021; Ringot *et al*., 2004).

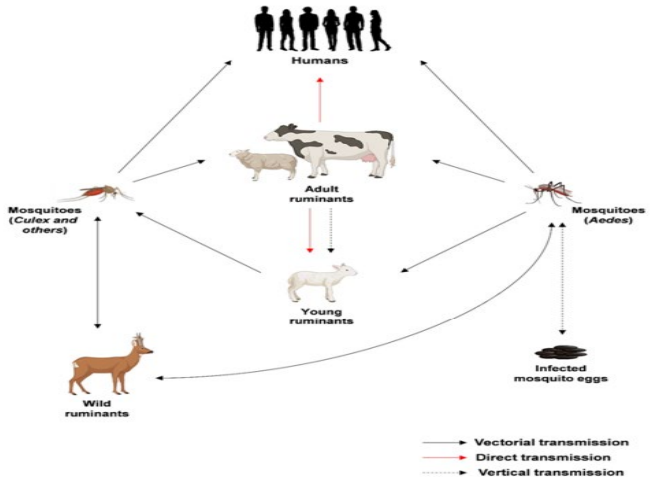


Figure 4: Epidemiological transmission cycles of RVF.

Source;(Lapa *et al*., 2024)

The virus can be maintained in an enzootic cycle involving Aedes mosquitoes which are able to transmit the virus vertically to their offspring. Epizootic outbreaks are often linked with unusual rains or warm seasons, favoring the hatching of infected Aedes eggs that are then able to initiate virus circulation. Subsequently, large numbers of secondary vectors belonging to the Culex genus could be infected and induce the emergence of epidemic/epizootic outbreaks. Transmission to humans occurs through direct contact with high virus loads when aborted fetuses are manipulated.

2.1.1.4. Prevention and control of Rift valley fever virus

Typically, a number of control measures are mentioned, such as vaccination of livestock, vector control with a focus on larvicides in vector breeding sites rather than aerial sprayings targeting adults, and control of livestock movements with regard to trade and export(Mhina *et al*., 2015). RVF persistence mechanisms should be characterized in order to support long-term surveillance programs, predict re-emergence, and evaluate the effectiveness of control measures (Figure 5). These persistence mechanisms in spatially heterogeneous systems involve both large-scale geographical and local factors, such as pathogen reintroduction from nearby regions. However, the effects of hosts, vectors and their environment on the persistence of RVF are not yet well understood or quantified. In order to improve understanding of how environmental factors and animal trade influence looking the transmission dynamics of Rift Valley fever is important (Tennant *et al*., 2021).

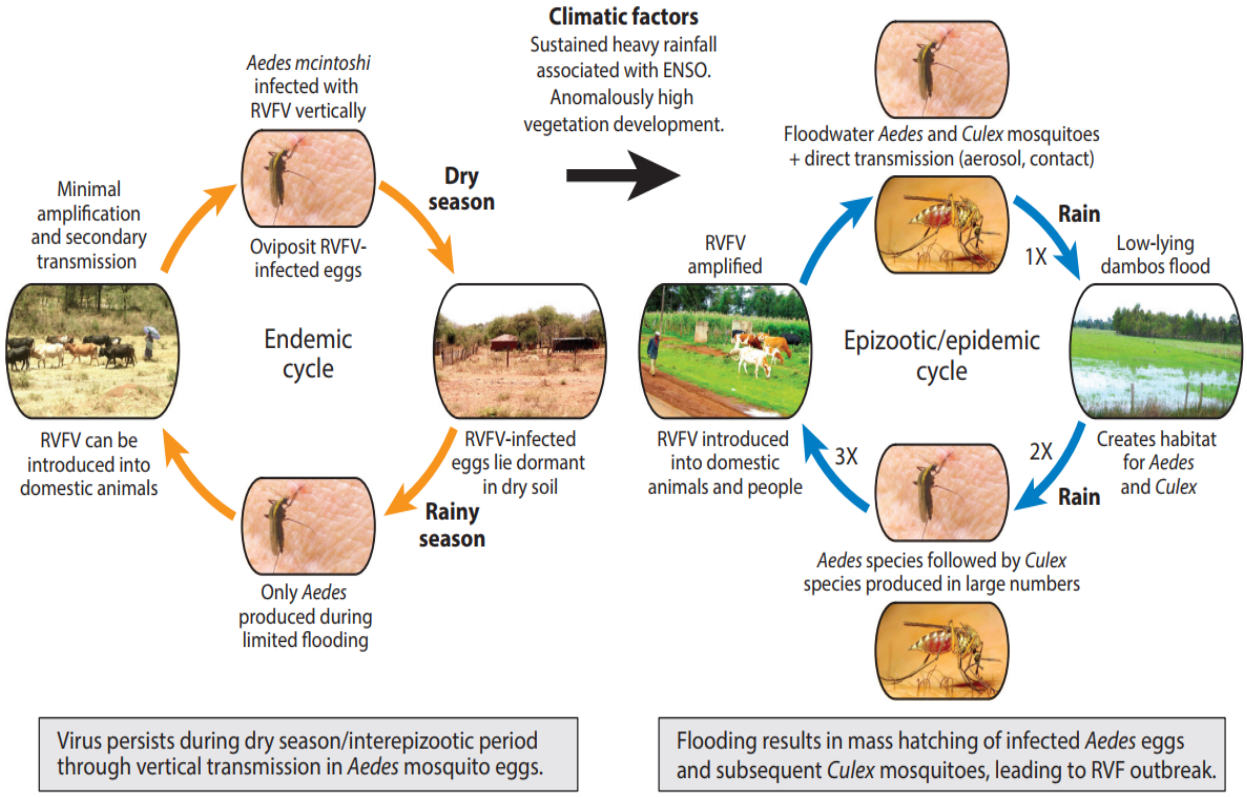


Figure 5: Schematic of the Rift Valley fever virus (RVF) life cycle

depicting the endemic (*left*) and epizootic/epidemic (*right*) phases, depending on the temporal and spatial extent of excessive rainfall. The 1X, 2X, and 3X labels in the epizootic/epidemic phase indicate key pathways that may be targeted by mosquito control measures, for instance, larviciding at 1X or 2X or adulticiding at 3X, for substantial reduction of RVF transmission. Abbreviation: ENSO, El Nino Southern Oscillation(Linthicum *et al*., 2016).

2.1.1.5. Diagnostic Techniques of RVF (OIE, 2018)

2.1.1.5.1. Serological tests

Samples collected from animals for antibody testing may contain live viruses and appropriate inactivation steps should be put in place. A combination of heat and chemical

inactivation has been described. Immunofluorescence assays are still used, although cross-reactions may occur between RVF and other phleboviruses. Techniques such as the agar gel immunodiffusion (AGID), radioimmunoassays, haemagglutination inhibition (HI), and complement fixation are no longer used. Several assays are available for detection of anti-RVF antibodies in a variety of animal species (RVF , 2018).

Currently the most widely used technique is the ELISA for the detection of IgM and IgG. Virus neutralization tests have been used to detect antibodies against RVF in the serum of a variety of species. Neutralization tests are the most specific diagnostic serological tests, but these tests can only be performed with live virus and are not recommended for use outside endemic areas or in laboratories without appropriate biosecurity facilities and vaccinated personnel. However, alternative neutralization assays not requiring handling of highly virulent RVF and not requiring high containment, are being developed and validated(Lvov *et al*., 2015).

2.1.1.5.2. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is a reliable and sensitive test to detect antibodies against RVF. Both IgG and IgM ELISAs are available for most species. IgM-capture ELISA allows diagnosis of recent infections. There are four major types of ELISA: Direct ELISA (antigen-coated plate; screening antibody), Indirect ELISA (antigen-coated plate; screening antigen/antibody), Sandwich ELISA (antibody-coated plate; screening antigen) and Competitive ELISA (screening antibody)

Direct ELISA

Both direct and indirect ELISAs begin with the coating of antigens to the ELISA plates. The first binding step involves adding antigens to the plates, which are incubated for one hour at 37 °C or can be incubated at 4 °C overnight. Once the incubation step is completed, the next step is to wash the plates of any potential unbound antibodies and block any unbound sites on the ELISA plate using agents like BSA, ovalbumin, aprotinin, or other animal proteins. This second step is crucial because it prevents the binding of any

non-specific antibodies to the plate and minimizes false-positive results. After adding the buffer, the plate is rewashed, and a selected enzyme-conjugated primary detection antibody is added. The plate is further incubated for one hour.

In a direct ELISA, the primary detection antibody binds directly to the protein of interest. Next, the plate is rewashed to remove any unbound antibodies. An enzyme, such as alkaline phosphatase (AP) or horseradish peroxidase (HRP), is added to the plate, which results in a color change. The color change of the sample occurs by either the hydrolysis of phosphate groups from the substrate by AP or by the oxidation of substrates by HRP. The advantages of using direct ELISA include eliminating secondary antibody cross-reactivity, and due to fewer steps, it is rapid compared to indirect ELISA. Its disadvantages include its low sensitivity compared to the other types of ELISA and its high cost of reaction.

Indirect ELISA

The steps of the indirect ELISA are identical to the direct ELISA, except for an additional wash step and the types of antibodies added after the buffer is removed. Indirect ELISA requires two antibodies: a primary detection antibody that sticks to the protein of interest and a secondary enzyme-linked antibody complementary to the primary antibody. The primary antibody is added first, followed by a wash step, and then the enzyme-conjugated secondary antibody is added and incubated. After this, the steps are the same as the direct ELISA, which includes a wash step, the addition of substrate, and the detection of a color change. The indirect ELISA has a higher sensitivity when compared to the direct ELISA. It is also less expensive and more flexible due to the many possible primary antibodies that can be used. The only major disadvantage of this type of ELISA is the risk of cross-reactivity between the secondary detection antibodies.

Sandwich ELISA

Unlike direct and indirect ELISA, the sandwich ELISA begins with a capture antibody coated onto the wells of the plate. It is termed a “sandwich” because the antigens are

sandwiched between two layers of antibodies (capture and detection antibodies). After adding the capture antibody to the plates, the plates are then covered and incubated overnight at 4 °C. Once the coating step is complete, the plates are washed with PBS and then buffered/blocked with BSA. The blocking step is carried out at room temperature for at least 1 to 2 hours. Finally, the plate is washed with PBS once again before the antigen is added. The antigen of interest is added to the plates to bind to the capture antibody and incubated for 90 minutes at 37 °C.

The plate is rewashed, and the primary detection antibody is added to the plate and incubated for another 1 to 2 hours at room temperature, followed by a buffer wash. Then, the secondary enzyme-conjugated antibody is added and incubated for another 1 to 2 hours. The plate is rewashed, and the substrate is added to produce a color change. The sandwich ELISA has the highest sensitivity among all the ELISA types. The major disadvantages of this type of ELISA are the time and expense and the necessary use of “matched pair” (divalent/multivalent antigen) and secondary antibodies.

Competitive ELISA

The competitive ELISA tests for the presence of an antibody specific for antigens in the test serum. This type of ELISA utilizes two specific antibodies: an enzyme-conjugated antibody and another antibody present in the test serum (if the serum is positive). Combining the two antibodies into the wells will allow for competition for binding to antigens. The presence of a color change means that the test is negative because the enzyme-conjugated antibody bounds the antigens (not the antibodies of the test serum). The absence of color indicates a positive test and the presence of antibodies in the test serum. Competitive ELISA has low specificity and cannot be used in dilute samples. However, the benefits are that there is less sample purification needed, it can measure a large range of antigens in a given sample, it can be used for small antigens, and it has low variability.

2.1.2. West Nile Fever Virus

West Nile (WN) virus, the causative agent of West Nile fever, a dengue-like infection in humans, is one of the most widely distributed arthropod-borne viruses extending, until recently, from Africa, the Middle East, Europe, and western Asia. WN virus is a natural avian virus transmitted between birds primarily by ornithophilic mosquitoes, although isolations have been reported from mammals and amphibians, other mosquitoes, and ticks. This virus has one of the broadest host and vector ranges and historically caused clinical disease in humans and equines only(Bosco-Lauth & Bowen, 2019).

2.1.2.1. The organism

West Nile virus is a positive-stranded RNA virus in the family Flaviviridae (genus *Flavivirus*). It belongs to the Japanese encephalitis virus complex or serogroup. The two most common genetic lineages of WNV are lineage 1, which contains 3 clades (1a, 1b and 1c), and lineage 2. Both lineages contain virulent viruses, as well as strains that usually cause asymptomatic infections or mild disease. Many of the virulent viruses from recent outbreaks belonged to clade 1a, which is widespread(Byas & Ebel, 2020).

West Nile and *Japanese encephalitis* viruses are members of the *Japanese encephalitis* serological group of the genus *Flavivirus* and therefore closely related genetically and antigenically. They share several properties, including the use of birds as their major wildlife maintenance host and Culicine mosquitoes for transmission, and they are both associated with severe human disease, as well as fatal infections in horses. The emergence of these two viruses, and their well-established propensity to colonize new areas, make it timely to re-examine their ecology, biology, molecular structure, replication and epidemiology(Oluwayelu *et al*., 2018).

The virion consists of an envelope surrounding an icosahedral capsid of approximately 50 nm in size. The ~11 kilobase genome encodes a single open reading frame, which is flanked by 5′ and 3′ untranslated regions (UTR). The approximately 3000 amino acid polyprotein is cleaved into ten proteins by cellular and viral proteases. Three of these proteins are the

structural components required for virion formation (capsid protein (C)) and assembly into viral particles (premembrane (prM) and envelope proteins (E)). The other seven viral proteins are nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) and are all necessary for genome replication. NS3 contains an ATP-dependent helicase, and in conjunction with the NS2B protein, a serine protease, which is required for virus polyprotein processing. NS5 is a methyltransferase and RNA-dependent RNA polymerase (NS5). The other NS proteins are small, generally hydrophobic proteins of disparate functions(Rossi *et al*., 2010).

2.1.2.2. History

The West Nile Virus (WNV) was first found in a febrile adult woman in the West Nile District of Uganda in 1937. The virus became recognized as a cause of severe human meningoencephalitis (inflammation of the spinal cord and brain) in elderly patients during an outbreak in Israel in 1957(Lanciotti *et al*., 1999) and now a days the virus is present in every continent, except Antarctica.

West Nile virus historically was largely confined to Africa, the Middle East, and parts of Russia, India, and Indonesia, where it caused occasional, usually minor, epidemics of dengue like illness or sporadic encephalitis. However, the virus eventually was imported more broadly into Europe by migratory birds, and in 1999 it reached the United States, emerging in New York City, where it was detected in both birds and people. The virus subsequently was isolated from mosquitoes in Connecticut, and antibodies were detected in horses in Connecticut and New York state. West Nile virus also spread into Canada and the Caribbean and, later, to most other U.S. states(Datta *et al*., 2015).

First isolated from a woman in the West Nile district of Uganda in 1937(Lanciotti *et al*., 1999), is today found commonly in many countries including Ethiopia. It was identified in birds (crows and Columbiformes) in Nile delta region in 1953(WHO/FAO *et al.,* 2009). Before 1997 WNV was not considered pathogenic for birds, but at that time in Israel a more virulent strain caused the death of different bird species presenting signs of

encephalitis and paralysis(Kilpatrick *et al*., 2007). Human infections attributable to WNV have been reported in many countries in the World for over 50 years(Mohammed *et al*., 2023).

In 1999 a WNV circulating in Israel and Tunisia was imported in New York producing a large and dramatic outbreak that spread throughout the continental United States of America (USA) in the following years. The WNV outbreak in USA (1999-2010) highlighted that importation and establishment of vector-borne pathogens outside their current habitat represent a danger to the world(Klingelhofer *et al*., 2023).

The largest outbreaks occurred in Greece(Ulbert, 2011), Israel(Santos *et al*., 2022), Romania(Oluwayelu *et al*., 2018), Russia(Fischer *et al*., 2021) and USA(Leta *et al*., 2018). Outbreak sites are on major bird's migratory routes. In its original range, WNV was prevalent throughout Africa, parts of Europe, Middle East, West Asia, and Australia. Since its introduction in 1999 into USA, the virus has spread and is now widely established from Canada to Venezuela(Braack *et al*., 2018).

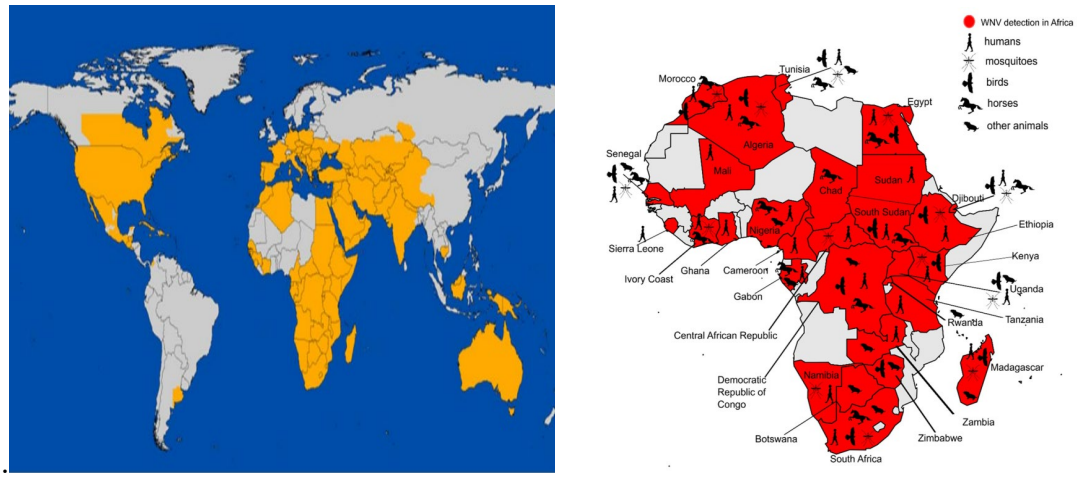
2.1.2.3. West Nile Fever Distribution and Transmission Cycles

West Nile Virus has been isolated from several species of birds and mammals. Wild birds are considered the primary disease reservoir for WNV and may reintroduce the virus during annual migrations(Lvov *et al*., 2015). Infections in mammals often fail to produce virus levels that are high enough to infect mosquito vectors. WNV has been detected in many species of mosquitoes naturally, especially Culex spp. which are primarily bird feeders(Troupin & Colpitts, 2016).

Vector competency studies clearly implicate mosquitoes in the transmission of WNV to humans(Benjelloun *et al*., 2016). Studies have shown in laboratory settings, WNV can replicate from 14°C in mosquitoes up to 45°C in some avian hosts. WNV replicates quickly in mosquitoes when temperatures exceed 25°C, however, temperatures above 30°C have slowed the growth and replication of WNV in the vector species *Culex univittatus*(Paz, 2015). Precipitation has played a key role in WNV incidence in the USA as well. One study

showed that in weeks with one or more days of precipitation of 50mm or higher there was shown to be a 33% in WNV infections reported that week and in the following two weeks. Infected mosquitoes can transmit WNV for life(Petersen *et al*., 2013).

Before 1999, WNV was limited to Africa, with occasional outbreaks in the Mediterranean region, Romania and Russia (Figure 6). The migration of people, birds and mosquitoes from Africa and Asia led to the establishment of vectors, hosts and the virus in Europe and North America. Initially, multiple species of the *Culex pipiens* complex adapted to both temperate and tropical climates. Many of these mosquito species, such as *C. tarsalis,* subsequently successfully adapted to multiple agricultural and urban landscapes. In Africa and Asia, multiple species of mosquitoes and birds have been associated with the maintenance of WNV infection and transmission to horses, with very little disease in birds or horses(Helmy *et al*., 2017).



A B

Figure 6: Map of West Nile virus distribution globally, yellow (A) and in Africa (B) based on sero-epidemiological surveys carried out on humans and animals, and viral isolation in mosquitoes.

Source;(Mencattelli *et al*., 2022)

2.1.2.4. Transmission Cycle

WNV is an avian zoonosis, being maintained in nature by transmission among ornithophagic Culex mosquitoes and a wide variety of birds (Figure 7), especially those in the order Passeriformes. Several Culex species have been implicated as vectors based mostly on laboratory vector competence studies, including Culex *univittatus*, Culex *neavei* and perhaps the Culex *pipiens* complex in Africa, Cx. *pipiens* complex and perhaps Culex *modestus* and Culex *perexiguus* in Europe, Culex *annulirostris* and perhaps the Cx. *pipiens* complex in Australia, and Culex *bitaeniorhynchus*, Culex *vishnui*, Culex *pseudovishnui*, Culex *tritaeniorhynchus* and Culex *quinquefasciatus* in India and Pakistan. However, the exact role of these species in virus epidemiology has been confusing due to their frequent blood feeding on large mammals and the limited numbers of isolations made during outbreaks and ecological investigations(Reisen, 2013).

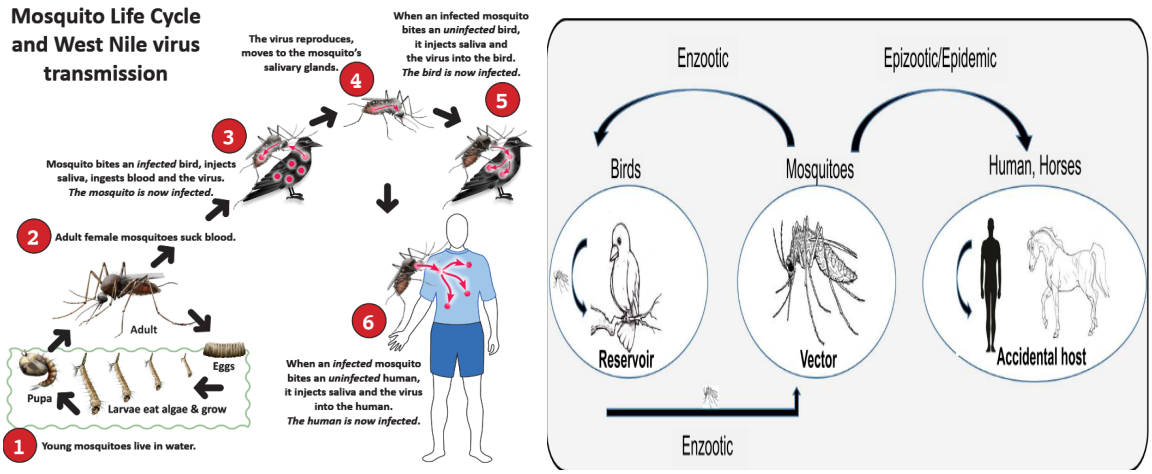


Figure 7: Transmission cycle of West Nile Fever virus,

source:(Dabbu Kumar *et al*., 2018; Kilpatrick *et al*., 2007;Brault, 2009; Andrea & Colpitts, 2016)

2.1.2.5. Host range of West Nile Fever Virus

Vertebrate hosts susceptible to WNV are birds, reptiles, mammals, and humans. Wild birds, such as Passeriformes (notably house sparrows and corvids) are reservoirs, amplifying

WNV, and sustain a high titer viraemia. Most diurnal birds of prey (order Accipitriformes) and Owls (order Strigiformes) show a predisposition to infection and high disease prevalence. RNA persists in hosts long-term, and some species show poor virus neutralization(Artsob *et al*., 2009).

2.1.2.6. West Nile virus infection in Human

In humans, infection with West Nile virus causes a flulike illness known as West Nile fever(F. J. May *et al.,* 2011). Most human infections are inapparent or mild, with illnesses that usually lasts only a few days. However, in a minority of infected persons, particularly in those over age 50, the virus multiplies in the lymphoid tissue and circulates in the bloodstream (possibly also multiplying in leukocytes, or white blood cells) before penetrating the central nervous system (CNS)(Garcia-Carrasco *et al*., 2023; Garcia-Romero *et al*., 2023).

In the CNS, depending on which tissues are infected, the virus can give rise to any of various conditions classified as West Nile neuroinvasive disease. In some instances, the virus reaches the brain, resulting in West Nile encephalitis, symptoms of which include headache, fever, neck stiffness, disorientation, and muscle weakness. Death may result(Benjelloun *et al*., 2016). Alternatively, spread of the virus to the CNS can cause West Nile meningitis, a distinguishing symptom of which is stiff neck, or West Nile poliomyelitis, a syndrome characterized primarily acute flaccid paralysis(Chowers, 2009).

2.1.2.7. West Nile virus infection in Animals

West Nile virus has a wide host range and has been detected in at least 48 species of mosquitoes(Barzon *et al*., 2015), 320 species of birds(Sule *et al*., 2018), 2 species of reptiles(Habarugira *et al*., 2020), and 25 mammalian species(Ulbert, 2011). The virus is mainly maintained by bird–mosquito–bird cycle, and corvids (eg, crows) are the main amplifying host(Garrigós *et al*., 2023).

2.1.2.7.1. Birds

During the introduction of West Nile virus to the United States, mortality observed in the Bronx zoo and surrounding areas showed that avian species across multiple orders could be affected and included common crows, a fish crow, black-billed magpies, a black-crowned night heron, laughing gulls, a mallard duck, Himalayan Impeyan pheasants, a Blyth's tragopan, Chilean flamingos, guanay cormorants, bronze-winged ducks, a northern bald eagle and a snowy owl(Barbachano-Guerrero *et al*., 2019).

Lesion variability observed between species is likely multifactorial and related to host factors and intrinsic viral factors which depend on virus strain. Specifically, levels of high viremia associated with being an amplifier host have been shown to correlate with mortality in some birds and most major organ systems have been shown to be affected in natural avian WNV infections(Gamino & Hofle, 2013).

Neurological manifestations are indicative of viral encephalitis and are similar to findings seen in humans and other non-host vertebrate species(Gamino & Hofle, 2013; Fiacre *et al*., 2020; Mada *et al*., 2020). Histologically, there is perivascular cuffing, glial nodules and gliosis, neuronal necrosis, and occasional hemorrhage. Frequently affected regions of the nervous system include the brain stem and gray matter of the spinal cord, cerebellum, and thalamus. In addition to the nervous system, myocarditis is a common lesion in birds(Artsob *et al*., 2009; Benjelloun *et al*., 2016; Venter & Swanepoel, 2010).

Birds usually do not show any clinical signs when infected with WNV. Chickens can be infected with WNV and not become sick. However, natural diseases due to the virus have been reported in domestic geese, ducks, pigeons, and chickens. No domestic birds have been found in Connecticut carrying the disease(Castillo-Olivares & Wood, 2004).

There have been no cases of indoor pet birds becoming infected. It is also important to point out that birds are not currently suspected of transmitting WNV to humans. Regardless, gloves should be worn if handling any dead bird or animal. In general, birds that are susceptible to West Nile virus–associated disease can exhibit a variety of

nonspecific (eg, emaciation, dehydration, feather loss, weakness, recumbency, drooped head, anorexia, lethargy, fluffed feathers) and neurologic signs (eg, ataxia, head tilt, nystagmus, tremors, hind limb paresis, seizures, blindness)(Athanasakopoulou *et al*., 2023; Llorente *et al*., 2023).

Younger birds of many species are more susceptible to disease, exhibiting higher viremia titers than do more mature (ie, adult) birds. For example, very young (eg, 1-day post-hatch) chickens have succumbed to experimental WNV infection after exhibiting fluffed feathers, weakness, and lethargy. However, older chicks and adult chickens and turkeys are not known to develop clinical signs of WNV-associated disease and are likely resistant(Sambri *et al*., 2013).

Many healthy WNV-seropositive chickens have been observed, and experimental infections have failed to induce disease. Among other domesticated or semi-domesticated fowl, some breeds of geese and ducks are clinically affected by WNV infection. The most susceptible wild birds belong to the corvid family, and numerous raptor species (eg, owls, hawks, falcons) are also susceptible(Hammami *et al*., 2017).

2.1.2.7.2. Horses

Clinical signs of WNV infection in horses, aside from fever, are primarily related to nervous system infection and inflammation. Approximately 20% of infected horses develop clinical neurological signs(Barzon *et al*., 2013). Mortality in unvaccinated horses is between 30 and 50%, inclusive of both natural death and elective euthanasia. The most severe clinical signs in horses include limb ataxia, tetra paresis, paraparesis, recumbency, seizures and death. Additional signs include cranial nerve deficits, muscle fasciculations, hyperexcitability and behavioral changes(Abdullahi *et al*., 2020).

The cerebral cortex seems to be least affected. Extra neural disease in horses includes sporadic renal hemorrhage, lymphoid atrophy, and myocarditis. Several commonalities unite the clinical presentation of WNV in the most affected vertebrates: birds, horses, and

humans. These include neurotropism characterized by primarily mononuclear inflammation, neuronal necrosis and gliosis which frequently affects gray matter and varies according to host and virus strain. In addition, renal and ocular tropism seems to be a conserved aspect of clinical disease in birds and humans(Ozcelik *et al*., 2023).

2.1.2.7.3. Additional Affected Vertebrate Species

In addition to birds, humans, and horses, WNV infects and causes disease in an extraordinary array of vertebrate species(Byas & Ebel, 2020). In many of these, clinical disease is solely neurological and thus like what is seen in humans and horses. Histologically, these animals had non-suppurative meningoencephalitis which frequently and preferentially affected the gray matter of the brainstem and spinal cord. Similarly, convulsions and ataxia in multiple WNV-infected sheep were seen in association with lymphoplasmacytic meningoencephalitis and myelitis characterized by perivascular cuffing and necrosis(Artsob *et al*., 2009).

While the expected neurological disease is common in dead-end hosts, some species and individual animals have unique disease presentations. In addition to encephalitis, fox squirrels experience myocarditis, which has been mentioned as a common manifestation in birds. In alligators infected with WNV, systemic disease is sometimes accompanied by inflammatory nodules in the skin composed of lymphocytes and macrophages. In a case report of a dog, polioencephalomyelitis and myocarditis were accompanied by vasculitis, pancreatitis and plasmacytic synovitis (inflammation of the articular synovial surface)(Byas & Ebel, 2020; Eybpoosh *et al*., 2019).

West Nile virus infections have been documented in sheep(Rimoldi *et al*., 2017), cattle (Barzon *et al*., 2013) and pigs(Sargeant & O'Connor, 2020) in Africa and Eurasia. Most infections were without clinical signs and animals developed antibodies to the virus. However, in laboratory studies (Table 7) sheep infected with WNV exhibited fever, abortion and encephalitis(Rimoldi *et al*., 2017) (Table 7).

Table 6: Laboratory tests used for WNV diagnosis and surveillance in humans, equines, birds, and mosquitoes.

Sample/method	Human	Equine	Bird	Mosquito
Specimen	• Serum • plasma • CSF and tissue	• Serum and tissue	• Serum, tissue and oral swabs	• Mosquito pools
Indirect detection	ELISA (IgM, IgG, IgG avidity) • IFA • Sero-neutralization (PRNT)	ELISA (IgM, IgG) • Sero neutralization (PRNT)	Domestic fowl: ELISA (IgM, IgG); • Wild birds: Competition ELISA, or indirect ELISA with commercial anti multiple species conjugate; • Sero neutralization	
Direct virus detection	RT-PCR • Isolation in cell culture • Immunohistochemistry	RT-PCR • Isolation in cell culture • Immunohistochemistry	Rapid immunochromatographic tests • RT-PCR • Isolation in cell culture	Rapid immunochromatographic tests; • RT-PCR • Isolation in cell

Source;(European Centre for Disease Prevention and Control, 2013)

2.1.3. Pastoralism and the role of livestock in Ethiopia

Livestock is central to the livelihoods of poor communities in sub-Saharan Africa, both in rural and in urban settings(Alemayehu *et al*., 2021). In high-potential rural areas, livestock generally is part of an integrated mixed crop-livestock production system, while in low potential areas, usually lowland areas, livestock is at the core of the agropastoral and pastoral systems. Livestock bring multiple benefits to the poor. The first is the provision of high-quality food. There is growing evidence that livestock keeping households consume more animal-sourced food, have healthier diets and an increased well-being compared to those without livestock(Alemu *et al*., 2023).

Additionally, livestock generate income and often bring cash needed for health care, school fees, and agricultural inputs. It provides manure, which in the mixed crop-livestock system is often the only fertilizer available, as well as labours for ploughing and threshing. Finally, livestock serve as a financial instrument as well as contributing to social status. When implemented in the right way, the livestock revolution could increase wellbeing of the rural poor across the continent.

Pastoralism is a traditional livelihood system based primarily on livestock production for subsistence and characterized by different degree of migration of the community in search of grazing pasture and water for their livestock. Pastoralism is a successful strategy to support a population on less productive land and adapts well to the environment(Behnke & Kerven, 2011).

In developing countries like Ethiopia, pastoralism accounts for the livelihoods of 50 – 100 million people and approximately 60% of this population lives in more than 21 African countries confined to the most arid regions of the continent. In East Africa, Ethiopia has the largest pastoralist population (7-8 million), representing around 20 ethnic groups and constitute around 14-18% of the total Ethiopian population. Pastoralists in Ethiopia are found in seven regions including Afar, Somali, SNNP, Oromia, Dire Dawa, Benshangul Gumuz and Gambella Regional States(Solomon, 2006).

The major ethnic groups in Ethiopia are Somalis, Afar, Kereyu and Borena pastoral communities occupying the Eastern, Northeastern, and southern lowlands of the country. Ethiopia's total livestock population has reached more than 88 million in head count, and is the largest in Africa(EEA, 2021). The livestock sub-sector contributes an estimated 12% to total GDP and over 45% to agricultural GDP. On average, the pastoral livestock population accounts for an estimated 40% of the total livestock population of the country and the sector plays a crucial role for livelihood of the pastoralist communities(CSA, 2021).

According to IGAD, the pastoral livestock population also contributes to transport services in pastoral areas and provides products such as milk, meat, skin and hides, also involved in livestock sharing networks as a collective insurance value, though the value of these components has largely been underestimated(Yismaw, 2021).

In general, considering all these values, IGAD estimated that the contribution of pastoral livestock to Ethiopia's GDP is very significant and exceeds 90 billion ETB, approximately US$10.6 billion in 2008/09. More than the economic value, to the pastoralist community livestock shares the core of their cultural identity, wealth ownership and means of subsistence during the hostile and challenging climatic and ecological settings of the arid zone. As in most developing countries, pastoralist in Ethiopia have been still marginalized in terms of basic infrastructure including access to basic education, health services to both human and their livestock, and access to communication despite some efforts in the past decades by government and non-government organization. Hence, the pastoralist livelihoods are highly vulnerable to the effect of climatic changes including the recurring drought and epidemics of diseases affecting livestock and humans with huge negative impact on their survival, animal productivity and public health of the pastoralist population(Adamu *et al*., 2021).

2.1.4. Epidemiological Status of RVF and WNV in Ethiopia

Limited research has been conducted in Ethiopia to assess the prevalence of Rift Valley Fever virus (RVF) and West Nile fever virus (WNV); however, a recent serological study revealed evidence of active viral circulation among pastoralist communities in the country's remote regions (Table 8). These findings underscore the potential risk of RVF and WNV transmission in these areas, highlighting the need for enhanced surveillance and public health interventions to mitigate outbreaks in both human and livestock populations. Further investigation is warranted to understand the epidemiological dynamics and ecological drivers of RVF and WNV in Ethiopia's pastoral ecosystems.

Table 7: Epidemiology of Rift Valley Fever and West Nile viruses in Ethiopia

Study Site	Species of host studied	Laboratory Technique	Prevalence	Source
Somali Region, Ethiopia	Cattle	competitive ELISA	17.9%	(Ibrahim *et al*., 2021)
	Sheep	competitive ELISA	7.4%	
	Goat	competitive ELISA	6.3%	
	Camel	competitive ELISA	42.6%	
	Human	competitive ELISA	13.2%	
Gambella Region, Southwest Ethiopia	Cattle	indirect ELISA	7.6%	(Asebe *et al*., 2020)
South Omo area, southern Ethiopia	Cattle	indirect ELISA	5.0%	(Endale *et al*., 2021)
Mid-Rift Valley, Borena and Segen Valley, Ethiopia	Aedes spp., Culex, Anopheles, and Mansonia	reverse transcriptase-PCR	0%	(Jaleta *et al*., 2022)

2.1.5. Socio-Economic Importance of Arboviruses

Pastoralist communities practice traditional extensive livestock production systems and have close contact with their livestock. The majority of these communities consume raw milk and assist animal deliveries with bare hands, which might increase the risk of zoonotic disease transmission(Tajudeen *et al*., 2022). Nevertheless, community awareness about

zoonotic diseases is reported to be low. The risky practices and low zoonoses awareness of the communities could lead to health and economic impacts caused by various zoonotic diseases(Zerga, 2015). The Horn of Africa is the largest livestock-marketing hub in Africa, with over 20 million nomadic and semi-nomadic pastoralists living in the Horn of African drylands(Behnke & Kerven, 2011).

The Horn of Africa livestock export industry reportedly accounts for about 1.5 billion USD. Annual livestock trade through only the ports of Berbera and Bosaso is estimated to be 400 million USD. Formal and informal livestock trade is common in the region, with the informal accounting for a large share. About 60-80% of animals exported through Berbera port originate from the Somali region of Ethiopia. Most Horn of Africa countries do not exercise their full potential of livestock trade, due to diverse reasons such as poor policies and strategies, governmental negligence, inefficiencies, poor infrastructure, poor veterinary services, livestock diseases, insufficient quarantine services and lack of diagnostic facilities. The major livestock importers of the region are the Gulf countries, especially Saudi Arabia and UAE(Tigoi *et al*., 2015).

In Ethiopia, livestock marketing contributes immensely to national GDP. As it is a land locked country, livestock exported from Ethiopia transit through either Djibouti or Berbera ports. There are three types of livestock markets in Ethiopia. Primary markets: these markets are small in size receiving less than 500 head of cattle per week. They are located in small villages, are not fenced and have no water or feed troughs. Main actors in these markets are primary producers, local consumers and butchers, Secondary markets: These are average in size, receiving about 500-1000 head of cattle per week. They have fences, shade, water and feed troughs. They are in larger cities, for example, capital cities of regional states. The main actors are traders and butchers, but they also supply terminal markets, live animal exporters and meat processors and terminal markets: These are located in large urban centers, like the country capital and other larger cities. They receive more than 1000 head per week. The actors are mainly medium to large-scale traders and butchers(Nin-Pratt *et al*., 2009).

2.1.6. Multidimensional impacts of Rift Valley fever

By impacting livestock and human health, RVF also represents a multidimensional socio-economic threat. The socioeconomic consequences vary with the country and depend on the importance of pastoralism in the country's economic system. For example, the 2007 outbreak of RVF in Kenya induced a 48% drop in national production compared to the 14% loss caused by the outbreak of RVF in Yemen in 2000. Pastoralism plays an important role throughout Africa and is crucial for some economies like in the Horn of Africa. For example, in Kenya, livestock represents 90% of income and in Sudan, the livestock sector employs 40% of the population(Tinto *et al*., 2023).

At the individual and household scale in pastoral communities, livestock is the main source of both income and food (red meat and milk). Thus, livestock mortality and abortions represent a significant monetary loss for individual producers by reducing their activity and meat trade in the short term. The secondary effects linked to the presence of the disease can last for several generations of animals by disturbing herd dynamics thereby becoming a long-term problem. At community scale, disruption of red meat markets and of the milk trade can disturb all the livestock marketing chains by stopping or delaying sales, and hence affecting all the actors of the downstream marketing chain including producers, slaughterhouses, traders, and butchers, but also associated non-agricultural sectors(Tinto *et al*., 2023).

Indeed, on a community scale, the occurrence of RVF leads to a reduction in rural livelihoods and in the value of agricultural products (crops, milk and meat, animal, fruits, vegetables), and affects large parts of the local economy. In Kenya, a cross-sectional household survey showed that 70–92% of pastoral households depend on the income they get from the sale of livestock. Linked to the disruption of the community-based economy, RVF outbreaks also have an impact on the national and global economy. Indeed, disruption of the livestock marketing chain and the fall in the sale of red meat and derived animal products also impact urbanized areas by reducing household supplies and interrupting livestock-related urban industry and auxiliary services: reduced activity, increased

unemployment, and decreasing incomes. These impacts on urbanized areas then lead to a decrease in non-agricultural sectors (trade, transport, tourism, petroleum) and finally to significant financial loss at national scale. Moreover, RVF results in bans on livestock trade not only at the national, but also at an international level for countries with international animal trade like in the Horn of Africa(Ng'ang'a *et al*., 2016).

Likewise, public health, healthcare and national RVF control measures have a significant impact on the country's economy. The cost of managing human and animal deaths, the care required by human and animal RVF (treatment, hospitalization, veterinary care) and the control measures (control of animal movements and trade, livestock vaccination strategies) entail significant costs for the national economies of the affected countries. The status of being endemic for RVF can involve long-term economic costs due to irregular and recurrent RVF outbreaks as well as to unexpected costs for disease control(Peyre *et al*., 2015).

2.1.7. Psycho-social impact and food security threat

The occurrence of RVF can lead to significant psycho-social distress in pastoral communities(Tinto *et al.,* 2023). Indeed, for RVF diseased households, grief over the death of family members or close relatives, the fear of death and of the loss of livestock and/or production have been described as important deleterious psychological factors. In addition, impoverishment and the decrease in rural household livelihoods were perceived by pastoral communities as major threats of RVF outbreaks. Not only diseased families feel psychological distress due to RVF threats, many individual inhabitants of pastoral areas, from livestock farmers to people in non-livestock sectors, also suffer from similar symptoms(Chengula *et al*., 2013).

For some communities, livestock farmers' status is associated with pride, prestige, and influence, and, during RVF outbreak, ruminants at risk temporally lose their economic, nutritional, and social value. Thus, in addition to the potential impact on family structure and impoverishment, means the social status of most livestock farmers in RVF epidemic

areas is seriously eroded resulting in psycho-social distress for pastoral communities(Sindato *et al*., 2012). Finally, the food security of populations is seriously affected during outbreaks of arboviral diseases (Figure 8). Associated with the drop in food production, loss of rural and urban household livelihoods, an increase in the price of alternative sources of meat to compensate for the lack of red meat, and disruption of the food system disruption caused the ban on animal trade leads to significant food insecurity and particularly, to malnutrition(Sindato *et al*., 2012). Reports on the RVF outbreak in Kenya in 2020 showed that the consumption of meat declined and that a high proportion of people did not reach the minimum dietary score and consumed insufficient protein rich food(Tinto *et al*., 2023).

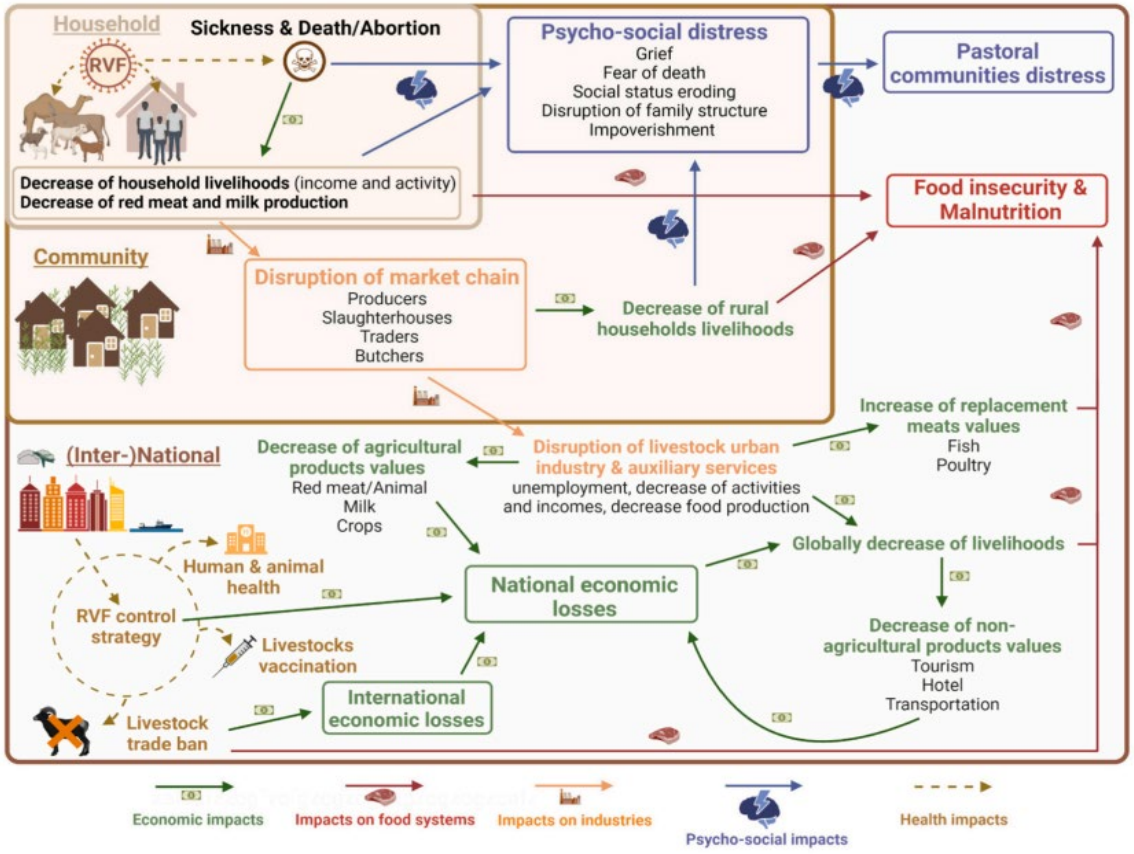


Figure 8: Schematic representation of the multidimensional socio-economic effects of RVF at household, community, and international scales

Source;(Tinto *et al*., 2023).

3. MATERIALS AND METHODS

3.1. Description of the Study Region and Districts

3.1.1. Study Region

Ethiopia is a landlocked nation in the Horn of Africa, borders Djibouti, Eritrea, Kenya, Somalia, South Sudan, and Sudan. Spanning over 1.1 million km², it is Africa's second-most populous country123 million (WB, 2022), with 80% rural residency. Administratively, it comprises 12 regional states and two chartered cities, subdivided into zones, districts (*woredas*), and sub-districts (*kebeles*), the latter averaging 5,000 rural or 25,000 urban residents (MoH/EHNRI, 2011). Ethiopia hosts Africa's largest livestock population: 65M cattle, 40M sheep, 51M goats, 8M camels, and 49M chickens (CSA, 2020). Its strategic Horn location near the Middle East bolsters international trade.

Ethiopia's pastoralists and agro-pastoralists predominantly inhabit lowland arid/semi-arid areas, covering 61% of the country's landmass, with 97% concentrated in the northeast, east, and south (Tofu *et al.,* 2023). The Afar region, the study area, hosts the second-largest pastoralist population (29%), after Somali region (53%) (Behnke & Kerven, 2011).

The Afar Pastoral Region is one of the twelve regions of Ethiopia and located in northeast of the country. The region land about 96,707 square kilometers is structured into five zones and 32 districts(political administration divisions), is situated between 39º 34' and 42º 28' East Longitude and 8º 49' and 14º 30' North Latitude(Shumbahri & Idris, 2021). The region shares borders with Tigray Region in the northwest, Amhara Region in the southwest, Oromia Region in the south, and Somali Region in the southeast. It also shares international boundaries with Djibouti in the east and Eritrea in the northeast. There are only three agroecological zones in the Afar region and the majority of households reside in the arid (48%) and semi-arid (49%) zones where pastoralism is the only livelihood option; only 3% live in the warm semiarid zone where there is sufficient irrigation water from Awash River to boost crop production to ensure food supply in the Region and in the country at large(Behnke & Kerven, 2011). Rainfall is bimodal throughout the region, with

a mean annual rainfall below 500 mm in the semi-arid western escarpments and decreasing to 150 mm in the arid zones to the east. The altitude of the Region ranges from 120 m below sea level in Danakil depression to 1500 m above sea level. Temperatures vary from 20°C in higher elevations to 48°C in lower elevations.

The Afar region has an estimated population of 1.9 million people projection on the 2007 Census (CSA, 2020), approximately two percent of the total Ethiopian population and the population density of the region is estimated to be 14.6 persons per square kilometer though it varies from zone to zone (CSA, 2020).

The major livelihood of the rural community in the region is livestock rearing which accounts for about 90% with limited irrigation agriculture along the river basins and low-lying riverine areas. The Afar community engages in subsistence livestock production for its economic, social, and cultural values. The region has 1,959,185 cattle 4,476,485 sheep, 8,843,082 goats, 1,258,971 camel and 308,835 equine populations (CSA, 2021).

The Afar Region has two major rivers (Awash and Telalak Rivers) which form their river basin. The Awash River which the major basin forming river originating from central Ethiopian highlands, flows across the region from south to north to enter to Lake Abe at the Djibouti border. The bank of Awash River is the main site where livestock migrate during dry seasons of the year. The Awash River basin is divided into Upper Awash Basin (from Koka Dam in central Ethiopia to Awash Station in Awash district and has altitude between 1500- 1000masl); Middle Awash Basin (from Awash Station to Gewane town which has an altitude between 1000-500masl) and Lower Awash Basin (from Gewane town to Lake Abe which has altitude below 500masl).

For this PhD research Zone Three (Gebiresu) was selected purposely for its accessibility and occurrence epidemiological suitable risk factors for Arboviruses and its vector. From this zone two districts (Amibara and Haruka districts) (Figure 9) were selected based on the population of livestock, human population density, accessibility to their respective subdistricts, existence of relative peace in the districts and presence of risk factors for the

occurrence of the Arbovirus infections. Based on CSA (2022) Zone-3 has 620,828 cattle, 1,089,703 sheep, 1,871,544 goat, 432,458 camel and 61,033 equines.

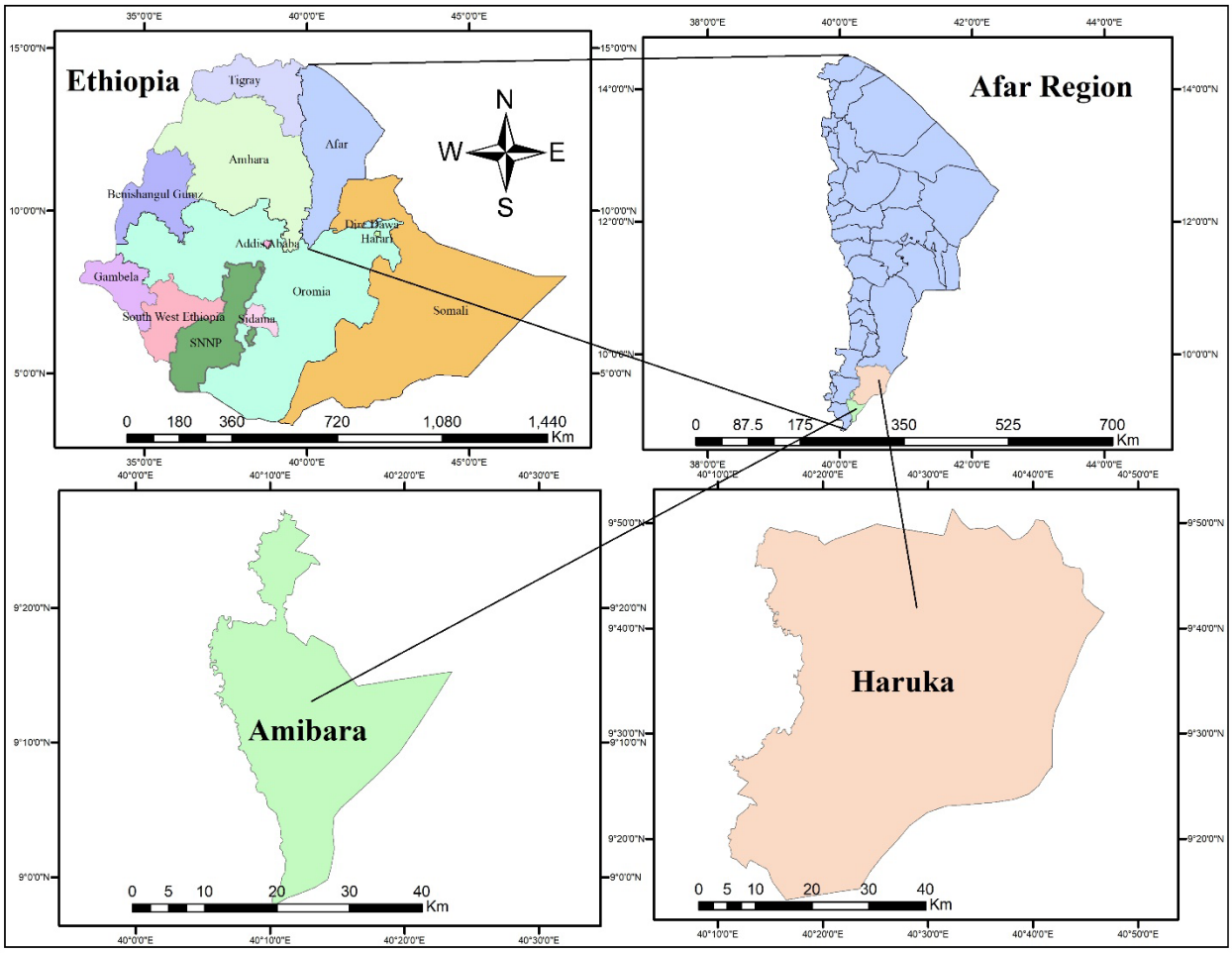


Figure 9: Map of the Study area, (ArcGIS 10.8. 2)

3.1.2. Study Districts

For the Epidemiology of Rift Valley Fever Virus and West Nile Fever Virus in Livestock (cattle, camel, sheep, goat, and donkey) Population and their Public Health Importance in Afar Region, Northeastern Ethiopia; Amibara and Haruka districts in Zone-3 (Gebiresu) of Afar National Regional State were selected.

A reconnaissance community-based survey and study site observation was conducted in June 2021 to generate information on RVF and WNV. Two districts (Amibara and Haruka) were selected based on the large number population of livestock, presence of water bodies; presence of large and medium scale irrigation activities, history of flooding; which favors the breeding and multiplications of Arbovirus vectors, Evidence of Abortion and Retained placenta, accessibility to their respective sub districts, existence of relative level of security in the districts and proximity to international borders.

3.1.1.1. *Amibara* District

Amibara District, situated in Zone Three of the Afar National Regional State, lies within the Middle Awash Basin approximately 260 km northeast of Addis Ababa. Bordered by *Awash Fentale* District to the south, the Awash River (separating it from *Dulecha* District) to the southwest, Administrative Zone 5 to the northwest, *Gewane* to the north, and the Oromia Region to the east, the area experiences high temperatures ranging between 25°C and 35°C. Annual precipitation averages below 600 mm, with the driest period (hagay) occurring in May/June, when arid conditions render bushes unsuitable for browsing. The primary rainy season (Karima), lasting from July to September, contributes over 60% of the yearly rainfall.

Comprising 11 kebeles (four urban and seven pastoral) the district's population totals 68,146, predominantly pastoralists. Ten kebeles have recently faced flood impacts. Key health facilities include Melka Were Health Center and Awash Arba Hospital, serving both local and neighboring communities. Livestock data (CSA, 2021) highlights 103,959 cattle, 122,526 goats, 48,043 sheep, 3,888 donkeys, and 39,995 camels. Camel ownership signifies wealth, with even a single camel elevating a household's status above poverty.

Pastoralists maintain mixed herds (camels, cattle, sheep, goats), migrating seasonally: wet periods are spent in the Halidege plains ("Adadi" pastureland), while dry seasons shift grazing to Awash Riverbanks. Floods during Karima displace communities from the river, and the district's vast pastures attract livestock from neighboring areas, fostering

intermingling of herds and species. Post-harvest private cotton farms also become high-traffic grazing zones (Figure 10), heightening risks of interspecies disease transmission. Additionally, the district hosts the Halaydage Wildlife Sanctuary and Awash National Park, where wildlife such as oryx, warthogs (Figure 11), gazelles, and zebras share grazing and watering sites with livestock. Frequent proximity between domestic cattle and wild animals underscores potential ecological and health interactions.

Figure 10: Large herd size and mixing of different species of livestock in Amibara district (*Halidegei Kebele*).

Figure 11: warthog grazing with cattle in Amibara district *Sidahfagei* sub-district

Figure 12: Animals and humans sharing drinking water from Awash River

3.1.1.2. Haruka district

Haruka District is one of the districts in Zone three of Afar National Regional State which is located in the Middle Awash Basin about 300km to northeast of Addis Ababa. The district was reorganized after 2019 as per the new restructuring of the district of Zone three. *Haruka* District is bordered on the south by Amibara District, on the west by the Awash

River, on the northwest by the administrative Zone 5, on the north by Gewane, and on the east by the Oromia Region. The area is characterized by high temperature; ranges from 25^0C to 35^0C. Usually the mean annual precipitation is less than 600 mm. The district has 9 *kebeles.* The district has a total human population of 20,146 of which the majority of the inhabitants are pastoralist. The livestock populations of the Haruka District were composed of cattle 21,269, goats 35,027, sheep 30,985, donkeys 1, 270 and camels 3,236.

Figure 13: Different groups of livestock shares same watering point (canal and stagnant water) in *Hassoba kebele.*

Figure 14: Livestock stayed the whole night under *woyane zaf* (*Prosopis juliflora*) in *Kalat Buri Kebele*

3.2. Research Approach

A mixed research approach was employed to collect both quantitative and qualitative data (Convergent Parallel Design), integrating the two forms of data, and using distinct designs that may involve philosophical assumptions and theoretical framework. The core assumption of using this form of inquiry is that the combination of qualitative and quantitative approaches provides a more complete understanding of a research problem.

3.3. Study design and study population

3.2.1. Epidemiology of Rift Valley fever virus and West Nile fever virus in the livestock

A cross-sectional study design was used to carry out on the sero-epidemiological detection of Rift Valley Fever Virus and West Nile Fever Viruses from 2021 to 2023. Convergent parallel mixed method was used to converge or merge quantitative and qualitative data in order to provide a comprehensive analysis of the research problem. In this design, collecting both forms of data at roughly the same time was carried out and then integrated the information in the interpretation of the overall results.

3.3.1. Study Populations

The Study population comprises of cattle, camel, goat, sheep and donkeys in the two districts of Afar Pastoral Region and sub-districts. For survey about the zoonotic importance of RVF and WNV was included in the study based on the inclusion criteria (livestock population ownership, and willingness of the pastoralists to participate in the research). All settlements (villages) in each selected sub-district were included systematically after obtaining the consent of elder's/clan leaders or sub-district administrators to participate in the study.

3.3.2. Sample Size Determinations

The sample size for epidemiology of RVF and WNV in the livestock and human population was determined according to Thrusfield (2005). Based on this, assuming the expected individual apparent seroprevalence of RVF of 17.9% (n=226) in cattle, 42.6% (n=375) in camels, 6.3% (n=91) in goats, 7.4% (n=106) in sheep and 13.2% (n=176) in humans as previous study was carried out in Somali region (Ibrahim *et al*., 2021), and for the determination of sample size in equines 9% (137) prevalence of West Nile Virus in Horses, sub-Saharan Africa, Djibouti was considered (Cabre *et al*., 2006) with a 5%, margin of error and 95% confidence interval and addition of 10% for the effectiveness and to mitigate

the influence of population characteristics in haphazard sampling and the total estimated sample size was 1,222 serum sample from livestock and volunteer humans were planned to take for serology analysis and virus isolation. The sample size was proportionally divided into two based on the livestock population and human population of the two districts. Initially, a sample size of 1,222 was planned for the study; however, only 945 serum samples were successfully collected. These comprised 199 samples from camels, 288 from cattle, 118 from donkeys, 155 from goats, and 185 from sheep. Due to constraints in ELISA kit availability, a subset of 736 samples was proportionally selected for screening, maintaining the relative distribution across species. Consequently, 155 camels, 224 cattle, 92 donkeys, 121 goats, and 144 sheep samples were analyzed to assess the presence of Rift Valley Fever virus (RVF) and West Nile virus (WNV)-IgM antibodies. This proportional allocation ensured representative testing across all species within the logistical limitations.

This study also aimed to assess the knowledge, attitudes, and practices (KAP) of farmers and health professionals. A structured questionnaire was administered to 150 participants during the study period, comprising 60 livestock owners (representing farmers), 50 public health workers, and 40 para-veterinarians and animal health professionals (representing health sector stakeholders). Data collection focused on evaluating their perspectives and behaviors related to zoonotic diseases to ensure comprehensive insights into sectoral challenges and practices.

3.4. Research Methods

3.4.1. Sampling Procedure and Method of Data Collection

After obtaining the local elders and owners' consent, the animals within the herd were selected using Haphazard sampling technique, because of lack of livestock registration in the study area. Based on the OIE/WOAH guideline, 5 ml blood sample was collected from selected livestock via the jugular vein puncture using a sterile plain and labelled vacutainer tube. To increase the amount of serum and maximum separation the test tubes were

centrifuged at approximately 8 minutes at 3000 rotations per minute and Sera were separated using Pasteur pipettes and placed in a labeled 1.8 ml Eppendorf /Nunk Cryo sera tubes and stored at -20˚C in *Melkawerer* agricultural research center. Sera samples were packed in cryovial rack box and transported on ice to Addis Ababa (AL-IHR) and stored at -20˚C until screened for antibody (IgG) against WNV, and RVF using competitive ELISA assay.

While shipping the serum samples, a marina cooler box with ice-frozen bricks was used to keep the cold chain, and samples were shipped to Aklilu Lema Institute of Health Research microbiology laboratory. 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 were identified by a temporary paper-based identification code and were recorded separately using a checklist prepared as a mini questionnaire at the time of blood collection.

3.4.2. Questionnaire Administration

Community-based cross-sectional survey was conducted in purposively selected pastoralists' sub-districts of the 2 districts as well as livestock and public health workers in the study areas. Prior to data collection, a list of all the sub-districts in the selected districts were obtained from the respective District livestock Office. Based on the number of households in each sub-district, the pre-estimated sample size (87) including Abattoir and Irrigation site workers were proportionally distributed. The required number of participants (husband or wife) was selected randomly from each sub-district using the lists.

Households whose livestock were sampled were questioned about livestock health and management as well as human demographic information, food consumption and animal contact practices. The respondents were asked about production system, raw milk, and meat consumption habit; abortion incidences for the last 6 months, management of aborted foetuses, zoonoses awareness (have you ever hear/know about zoonoses? With yes/no

response) some of the information was used to analyze the risk factors. The questionnaire was translated from English to Afar language before the interview.

The questionnaire included both closed (single response) and open (multiple responses) questions. A pre-test of the questionnaire was made and during pre-testing, additional information was gathered and some of the questionnaires were modified during preliminary survey in June 2021. Enumerators were recruited and trained to increase the accuracy of the responses. Accordingly, focus group discussions, semi-structured interviews and consultation meetings were conducted with pastoral elders and their council leaders to capture the existing pastoralists' livelihood. Individual interviews and groups discussions were further enriched and substantiated with an official consultation meeting with representatives of elders from all districts of the study areas.

The participants were interviewed in their local language by trained data collectors (degree holders and diploma graduate elementary school teachers) selected from the localities. Each interview was conducted by a house-to-house visit and at the health centers. Information on the socio-demographic characteristics of the participants was also included in the questionnaires. After completing the quantitative data collection, FGDs (with men and with women) comprised of 6-9 men or women who are not involved in the individual interview was conducted in the selected districts. Specific topics were prepared as guides for the discussion, moderated by the principal investigator and a trained animal health worker. The topics were presented one by one, allowing adequate discussion on each topic. The response was recorded using mobile phone recorder and a notebook, was translated into Amharic and then into English. The socio-demographic characteristics of the participants were recorded during the discussion. The data obtained was recorded in various data collection template applications designed for this survey purpose. Instruments like GPS and others were used during data collection for a timely and smooth acquisition of accurate data.

3.4.3. Data collection through observation

Collecting information on the presence or absence of wildlife species, on changes in habitat or land use area, the presence or absence of solid waste pollution, Movement of livestock, observation on vegetation cover and flooding or presence of stagnant water bodies help for mosquito breeding sites were employed using organized inspection checklists and observed data were supported with photos.

3.4.4. Serological Laboratory Analysis

Detection of anti-RVF-Nucleoprotein antibodies of Rift Valley Fever virus and West Nile virus in livestock population of Afar region (Amibara and Haruka) districts were carried out using the ID screen® Rift Valley Fever, these kits have a specificity of (99.58%-100%, n= 920) and a sensitivity of (91.24%-100%, n= 40). and West Nile competition multispecies ELISA kits (these kits have a specificity of (99.01%-100%, n= 384) and a sensitivity of (83.89%-100%, n=20) (ID-Vet Innovative Diagnostics, Montpellier, France). After allowing all the reagents and the sample to come to room temperature a pre-coated 96 well plate was used. After adding controls and test samples to the appropriate wells and tests for RVF were incubated at 37ºC ($\pm$2ºC) for 60 minutes (de Bronsvoort *et al.,* 2019) whereas West Nile was incubated at room temperature $\pm$5ºC for 90 minutes. Free components were washed away with a wash buffer. Horseradish peroxidase (HRP) conjugated detection reagents are used to visualize HRP enzymatic reactions. Tetramethylbenzidine (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 RVF/WNV-IgG amount bound on the plate. The results were read at an optical density (OD) of 450 nm using a 96-well ELISA plate reader (Multiskan™ FC Microplate Photometer) and was interpreted as positive or negative based on the manufacturer's recommended cut-off values. The test was validated according to the manufacturer's manual when the mean value of the negative control optical density (OD) (ODnc) was greater than 0.7 (ODnc > 0.7) and when the mean value of the positive control

OD (ODpc) was less than 30% of the ODnc (ODpc/ ODnc < 0.3) (de Bronsvoort *et al.,* 2019).

Then, the inhibition rate was calculated according to the following formula or using ID Soft™ software.

$$\frac{S}{N}(\%) = \frac{ODs}{ODnc} x100$$

With OD: optical density; nc: negative control; S: sample; S/N: competition percentage. S/N values lower than or equal to 40% were considered positive, values above 50% negative, and values greater than 40% and less than or equal to 50% are doubtful finally considered negative (Kainga *et al.,* 2022).

3.4.5. Quality control of data

The tools used for data collection and the subsequent verification processes carried out during and after data collection were employed to ensure the accuracy of the gathered data.

3.4.6. Data Processing and Analysis

Data processing and analysis was started in the field, with checking for completeness of the data and performing quality control checks, while sorting the data by instrument used and by group of informants. All collected data was cleared and entered into Microsoft excel and analyzed using R statistics software. In the study herds, age, sex, and species 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 and WNF. In this study, the outcome variables were ELISA IgG antibody positive and negative. Competitive ELISA was used for Rift Valley Fever and WNV identification in livestock. Based on manufacturer manual results were classified as seropositive and seronegative by calculating the mean OD value of each sample of the livestock. Results were expressed as percentage (Sample /Negative % = OD sample /OD NC x 100) and was interpreted as positive (S/N $\leq$40%) and negative (S/N > 40%).

The seroprevalence/apparent prevalence of IgG antibody elicited towards RVF and WNV was estimated by dividing the number of sampled animals with positive test results by the total number of tested animals. Here the livestock included in the study were apparently healthy and samples were 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, species, and site, was 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. A p-value below 0.05 was considered indicative of a statistically significant association at 95% confidence level.

For the survey, Pearson chi-square was used to evaluate the statistically significant of bivariate association of gender and selected covariate in each district. Bivariate and multivariable logistic regression analysis was performed to explore independent variables that were predictors of overall knowledge as well as that of the subscales of knowledge of RVF and WNV (sign/symptoms, mode of transmission, knowledge of effective treatment and preventive methods). Differences were considered significant when $p < 0.05$.

3.4.7. Ethical Considerations

The present study was a component of the thematic research project titled "Epidemiological Study on Major Mosquito-Borne Emerging and Re-emerging Viral Diseases of Humans and Animals in Selected Areas of Ethiopia," funded by the Vice President's Office of Research at Addis Ababa University, received ethical approval from the Institutional Review Committee (IRC) of the Aklilu Lemma Institute of Pathobiology. The Animal research ethics committee of the college of veterinary medicine and agriculture of Addis Ababa University has reviewed the research project and approved (VM/ERC/40/03/15/2023) all methods, including blood sample handling and collection. The animal owners gave their informed verbal consent for the collection of samples and

the ARRIVE criteria (Percie du Sert *et al*., 2020) were adhered to throughout the entire study process. All ordinary ethical considerations were followed with firm observation to the five degrees of animal welfares.

4. RESULTS

4.1. Description of Study Participants and Animals

From the 87 participants, 68.97% (60/87) were males, and 31.03% (27/87) were females. 91.95% (80/87) depend on subsistence farming for their livelihoods, while 8.05% (7/80) had other income-generating activities. The study site comprised two districts/woredas (Amibara and Haruka). The study focused on detecting IgG Rift valley and West Nile antibodies in 736 samples of camels (155), cattle (224), donkeys (92), goats (121), and sheep (144).

4.2. Seroprevalence of Rift Valley Fever Virus infection

A total of 736 serum samples were screened for IgG antibodies against RVF infection and the overall combined seroprevalence was 22.0% (162/736, 95% CI:19.41-24.79). The seroprevalence was significantly higher in goats (42.2%, 95% CI = 39.61-44.99, $p < 0.001$) followed by camels (30.97%, 95% CI = 28.38-33.76), cattle (14.3%, 95% CI = 11.74-17.09), sheep (21.5%, 95% CI = 18.91-24.29), and all the samples from donkey were negative (Table 8).

Table 8: Seroprevalence of RVF in different species of Livestock population

Species	No of animals sampled	No. positive (%)	95%CI	p-value
Cattle	224	32(14.3)	11.74-17.09	0.00
Goat	121	51(42.2)	39.61-44.99	0.00
Sheep	144	31(21.5)	18.91-24.29	0.001
Camel	155	48(30.97)	28.38-33.76	0.000
Donkey	92	0(0%)		
Overall	736	162 (22.0%)	19.41-24.79	0.001

4.2.1. Seroprevalence in different Sub-districts

The seroprevalence varied across sub-districts (Table 9). Animals from *Kealatburi* sub district had the highest seroprevalence (30.72%, 47/153) compared to *Halidegei* sub-district (22.4%, 51/228), *Sidahfagei* (19.2%, 37/193) and *Hassoba* (16.67%, 27/162) (p = 0.016). District seroprevalence ranged from 20.9% to 23.49%. The highest seroprevalence was observed in the *kealatburi* (30.72%, 95% CI = 28.48-33.13), with the lowest in *Hassoba* (16.67%, 95 CI = 14.43-19.08).

Table 9: Seroprevalence of RVF infection in different locations

Locations		Number of animals sampled	No. positive (%)	95%CI	p- value
District	Sub-district				
Amibara	*Halidegei*	228	22.4	20.16-24.81	0.017
	Sidahfagei	193	19.2	16.96-24.61	0.023
Haruka	*Hassoba*	162	16.67	14.43-19.08	0.002
	Kealatburi	153	30.72	28.48-33.13	0.001
Overall		736	22.0	19.76-24.41	0.013

4.2.2. Seroprevalence based on the Sex of the livestock population.

The overall seroprevalence was 22.16% in female animals and 21.43% in male animals, Furthermore, the overall seroprevalence across the species of livestock was higher in goats (58.33% in males and 38.14% in females) followed in camels (37.04% in males and 29.69% in females) (Table 10).

Table 10: Seroprevalence of RVF infection by sex of animals

Species	Sex	sampled animals	No. positive	Proportion (%)	95%CI	p- value
Cattle	Male	45	5	11.11	9.93-12.35	
	Female	179	27	15.08	13.9-16.32	0.546
Goat	Male	24	14	58.33	57.15-59.57	
	Female	97	37	38.14	36.96-39.38	0.544
Sheep	Male	27	5	18.52	17.34-19.76	
	Female	117	26	22.22	21.04-23.46	0.547
Camel	Male	27	10	37.04	35.86-38.28	
	Female	128	38	29.69	28.51-30.93	0.913
Donkey	Male	31	0	0		
	Female	61	0	0		
Overall	Male	154	33	21.43	20.25-22.67	
	Female	582	129	22.16	20.98-23.40	0.845

4.2.3. RVF seroprevalence at livestock herd and location level

The highest (73.68%) seroprevalence of RVF infection was observed in sheep from *kealatburi* sub-district followed by 65.71% and 48.57% of Goats from *halidegei* and *hassoba,* respectively ($p<0.001$). The seroprevalence of RVF infection was statistical difference ($p=0.001$) between camels (48.72%) from *kealatburi* and camel from *Sidahfagei* (27.5%) ($p<0.001$) (Table 11).

Table 11: Seroprevalence of RVF infection across locations and species

Species	Location	Sampled animals	No. positive	Prevalence (%)	95%CI	p-value
	Halidegei	63	10	15.9	14.15-17.71	
	Sidahfagei	72	10	13.9	12.15-15.71	0.017
Cattle	*Hassoba*	45	3	6.7	4.95-8.51	
	Kelatburi	43	9	20.93	19.18-22.74	
	Halidegei	35	23	65.71	63.96-67.52	
	Sidahfagei	24	7	29.17	27.42-30.98	0.023
Goat	*Hassoba*	35	17	48.57	46.82-50.38	
	Kelatburi	26	4	15.38	13.63-17.19	
	Halidegei	61	5	8.20	6.45-10.01	
	Sidahfagei	31	9	29.03	27.28-30.84	0.002
Sheep	*Hassoba*	32	2	6.25	4.5-8.06	
	Kelatburi	19	14	73.68	71.93-75.49	
	Halidegei	53	13	24.53	22.78-26.34	
	Sidahfagei	40	11	27.5	25.75-29.31	0.013
Camel	*Hassoba*	22	5	22.73	20.98-24.54	
	Kelatburi	39	19	48.72	46.97-50.53	
	Halidegei	15	0	0		
	Sidahfagei	26	0	0		
Donkey	*Hassoba*	27	0	0		
	Kelatburi	24	0	0		

4.2.4. Determining Potential Risk indicators

The study showed that the risk indicators for RVF seropositivity at individual livestock were location and species of livestock (Table 12; Table 13). Goats were (OR: 6.295, 95% CI = 3.716-10.460) six times more likely to be seropositive for RVF infection than cattle ($p = 0.001$). Livestock herds in *Sidahfagei* were (OR: .547, 95% CI = .333-.899) less likely to be seropositive for RVF infection than those in other areas ($p = 0.017$) by half.

Table 12: Univariable analysis of the relationship between Potential risk factors of RVF Sero-positivity

Variable	Level	No. examined	% Positive	95%CI	p-value
Species	Cattle	224	14.3	11.74-17.09	0.00
	Goat	121	42.2	39.61-44.99	0.00
	Sheep	144	21.5	18.91-24.29	0.001
	Camel	155	30.97	28.38-33.76	0.000
	Donkey	92	0		
Location	*Halidegei*	228	22.4	20.16-24.81	0.017
	Sidahfagei	193	19.2	16.96-24.61	0.023
	Hassoba	162	16.67	14.43-19.08	0.002
	Kealatburi	153	30.72	28.48-33.13	0.001

Table 13: Multivariable logistic regression analysis for potential risk factors associated with RVF in livestock.

Factor	Level	B	S. E	OR	95%CI	p-value
Species	Cattle	Ref.				
	Goat	17.913	.261	6.295	3.716-10.460	0.000
	Sheep	19.430	.259	2.704	1.063-4.298	0.000
	Camel	18.465	.272	1.175	0.343-1.7068	0.000
Location	*Halidegei*	Ref.				
	Sidahfagei	0.602	0.253	.547	.333-.899	0.017
	Hassoba	0.612	0.270	.542	.319-.921	0.023
	Kealatburi	0.914	0.296	.401	.224-.715	0.002

4.3. Seroprevalence of WNV infection

The seroprevalence of WNV infection was determined in 736 serum samples that were obtained from five different species of livestock populations (camel, cattle, donkey, goat, and sheep) that were located in two districts (Amibara and Haruka) of the Afar Pastoral region. Of 736 tested livestock serum samples, 50.7% (373/736) sera exhibited antibodies by competitive ELISA (95% CI: 47%–54.4%; P < 0.05), (Table 14). The seroprevalence is higher (p<0.05) in donkeys (76.1%) followed by camels (69%), cattle (52%), goats (34.7%) and sheep (25.7%) respectively. There is no statistical difference in the seroprevalence of West Nile fever infection by age category or sex of tested animals.

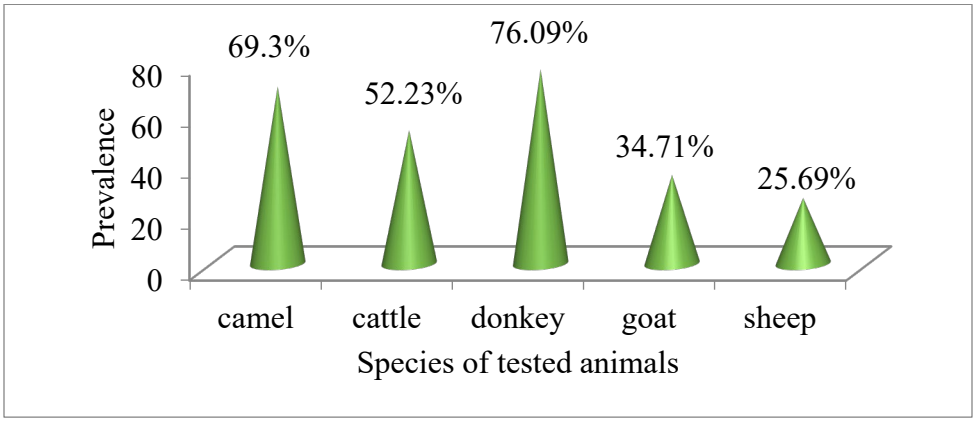


Figure 15: Seroprevalence of WNV in different species.

The study showed higher seroprevalence (figure 16) registered in young and older animals.

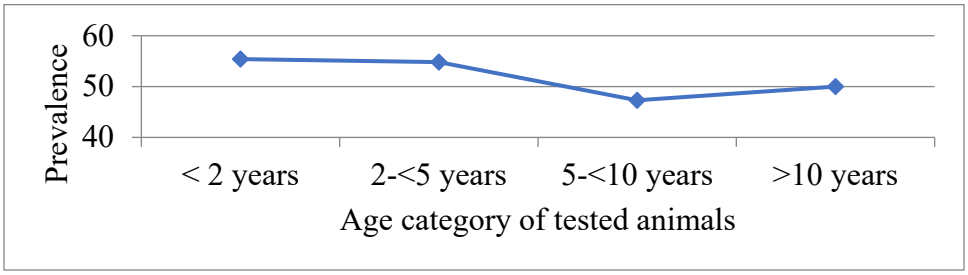


Figure 16: Distribution of WNV seroprevalence in different age category

Table 14: The seroprevalence of WNV in livestock populations across different variables

Variable	Category	No. of animals tested	No. Positive	Prevalence (%)	95%CI	$\chi 2$	P value
	Camel	155	107	69.03	60.2-75.4		
	Cattle	224	117	52.23	45.7-59.2		
Species	Donkey	92	70	76.09	65.7-84.2	93.171	0.000
	Goat	121	42	34.71	23.5-40.8		
	Sheep	144	37	25.69	20.2-35.3		
Sex	Female	582	298	51.2	46.6-57.0	0.305	0.322
	Male	154	75	48.7	39.5-55.3		
District	Amibara	421	216	51.3	46.7-57.2	0.155	0.694
	Haruka	315	157	49.8	37.5-53.5		
	<2 years	74	41	55.4	51.3-60.3		
Age	2-<5 years	208	114	54.8	50.9-57.1	3.629	0.092
	5-<10 years	332	157	47.3	44.2-52.4		
	> 10 years	122	61	50	47.8-55.1		
Overall		**736**	**373**	**50.7**	47–54.4		

Table 15: Univariable and Multivariable regression analysis of risk factors associated with West Nile virus infection seropositivity.

Variable	Category	No. of Sera tested	No. of Positive sera	Prevalence (%)	COR (95%CI)	AOR (95%CI)
Species	Camel	155	107	69.3	4.193 (2.528-6.955)	3.162(**1.973-5.067)**
	Cattle	224	117	52.2	2.039 (1.327-3.133)	1.515(1.038-2.212)
	Donkey	92	70	76.1	6.447 (3.888-10.688)	4.937(3.076-7.925)
	Goat	121	42	34.7	.701 (.389-1.261)	.477(0.275-0.828)
	Sheep	144	37	25.7	Ref.	Ref.
Sex	Female	582	298	51.2	1.105 (0.775-1.577)	1.174(0.802-1.718)
	Male	154	75	48.7	Ref.	Ref.
Age	<2 years	74	41	55.4	1.024(0.601-1.747)	.475(0.318-0.707)
	2-<5 years	208	114	54.8	1.385(0.835-2.298)	.576(0.401-0.828)
	5-<10 years	332	157	47.3	1.242(0.656-2.218)	.504(0.314-0.811)
	>10 years	122	61	50.0	Ref.	Ref.
District	Amibara	421	216	51.3	1.006(0.807-1.255)	1.086 (0.793-1.486)
	Haruka	315	157	49.8	Ref.	Ref.

3.1. Determining Potential Risk indicators for WNV

Table 16: Logistic regression analysis of the relationship between potential risk factors of WNV seropositivity

Variable	Category	No. examined	Positives	Prevalence	SE	OR	p-value
	Camel	155	107	69.03	Ref.	Ref.	Ref.
	Cattle	224	117	52.23	.258	.154	0.000
Species	Donkey	92	70	76.09	.233	.314	0.000
	Goat	121	42	34.71	.313	.105	0.000
	Sheep	144	37	25.69	.271	.634	0.094
Location	Amibara	421	216	51.3	Ref.	Ref.	Ref.
	Haruka	315	157	49.8	.161	.857	0.338

3.1. Seroprevalence of Rift Valley Fever and West Nile Fever Viruses Co-Circulations.

The present study revealed a 9.1%, 95% CI= (8.86–9.29) seroprevalence of co-circulation of RVF and WNV in the study area at the same time (Table 17).

Table 17: Seroprevalence of RVFV and WNV Co-Circulation in Livestock populations

Species	No of animals sampled	No. positive (%)	95%CI	p-value
Camel	155	32(20.7)	18.01-22.03	0.000
Cattle	224	16(7.14)	5.91-9.23	0.000
Donkey	92	0(%)		
Goat	121	14(11.6)	9.09-12.99	0.000
Sheep	144	5(3.5)	1.92-4.13	0.000
Overall	736	67 (9.1%)	8.86-9.29	0.000

0.000…statistically significant

3.2. Factors Associated with Co-Circulation of RVF and WNV.

There was a statistical difference in species and location for the seroprevalence of co-circulation. Camels showed a higher seroprevalence of 20.7% (32/155) compared to other animal species in the study area ($p \leq 0.001$). Additionally, the Amibara district exhibited a higher seroprevalence of 9.7% (41/421) (Table 18). Camels were seven times (OR: 7.016, 95% CI= 2.639–18.653) more likely to be seropositive for the co-circulation than sheep ($p \leq 0.001$). Livestock herds found in Amibara district were (OR: 1.165, 95% CI = 0.680–1.996) 1.2 times more likely to be seropositive for RVFV infection than those in Haruka areas ($p \leq 0.001$) (Tables 19 and 20).

Table 18: Factors associated with seroprevalence of antibodies against RVF and WNV for livestock populations of Afar Pastoral area, 2022.

Variable	Category	No of animals sampled	No. positive (%)	95%CI	Chi-Square	p-value
	Camel	155	32(20.7)	18.01-22.03		
	Cattle	224	16(7.14)	5.91-9.23		
Species	Donkey	92	0		41.616	0.000
	Goat	121	14(11.6)	9.09-12.99		
	Sheep	144	5(3.5)	1.92-4.13		
Sex	Male	155	13(8.4)	5.8.-10.9	.122	0.725
	Female	581	54(9.3)	6.03-12.01		
	<2 years	74	8(10.8)	7.1-11.9		
Age	2-<5 years	208	24(11.5)	9.02-13.09	3.60	0.308
	5-<10 years	332	28(8.4)	6.03-10.04		
	>10 years	122	8(6.6)	4.32-8.05		
Location	Amibara	421	41(9.7)	6.12-13.02	739.12	0.000
	Haruka	315	26(8.3)	7.02-10.01		

Camels were seven times (OR: 7.016, 95% CI = 2.639-18.653) more likely to be seropositive for the co-circulation than sheep (p =0.000). Livestock herds found in *Amibara district* were (OR: 1.165, 95% CI = .680-1.996) 1.2 times more likely to be seropositive for RVFV infection than those in Haruka areas (p = 0.000) (table 19).

Table 19: Binary logistic regression analysis of potential risk factors associated with anti-RVFV and WNV IgG seropositivity among livestock in the Afar pastoral area of Ethiopia 2022.

Variable	Category	No of animals sampled	No. positive (%)	Sig.	COR	95% CI	
						Lower	Upper
Species	Camel	155	32(20.7)	.000	7.016	2.639	18.653
	Cattle	224	16(7.14)	.000	2.056	.732	5.773
	Donkey	92	0				
	Goat	121	14(11.6)	.997	3.631	1.256	10.497
	Sheep	144	5(3.5)	.017	Ref.	Ref.	Ref.
Sex	Female	581	54(9.3)	.305	.981	.507	1.896
	Male	155	13(8.4)	.157	Ref	Ref	Ref
Age	<2 years	74	8(10.8)	.083	2.206	.737	6.603
	2-<5 years	208	24(11.5)	.301	2.207	.902	5.398
	5-<10 years	332	28(8.4)	.954	1.586	.662	3.800
	> 10 years	122	8(6.6)	.578	Ref	Ref	Ref
Location	Amibara	421	41(9.7)	.000	1.165	.680	1.996
	Haruka	315	26(8.3)	.000	Ref	Ref	Ref

COR, crude odds ratio; Ref., reference point

Table 20: Multivariate logistic regression analysis of potential risk factors associated with anti-RVFV and WNV IgG seropositivity among livestock in the Afar pastoral area of Ethiopia 2022.

Variable	Category	No of animals sampled	No. positive (%)	Sig.	AOR	95% CI	
						Lower	Upper
Species	Camel	155	32(20.7)	.000	7.302	2.757	19.339
	Cattle	224	16(7.14)	.001	2.166	.775	6.054
	Donkey	92	0				
	Goat	121	14(11.6)	.996	3.746	1.304	10.763
	Sheep	144	5(3.5)	.000	Ref	Ref.	Ref.
Location	Amibara	421	41(9.7)	.000	1.202	.706	2.045
	Haruka	315	26(8.3)	.000	Ref	Ref	Ref

Ref, reference point

3.2. Questionnaire survey of farmers

Livestock owners' knowledge and experiences regarding zoonotic infections were higher among those older than 30 years ($\chi 2$ =3.951, P =0.041). The questionnaire survey revealed that 62.1% of livestock owners perceived that diseases affecting livestock could be transmitted from animals to humans. Most of the owners (82.8%) were also aware of the potential risks associated with consuming raw food of animal origin such as raw milk or undercooked meat. However, none of the respondents had protocols for handling aborted foetuses and placental tissues to minimize disease transmission risks. Regarding prevention from vector mosquitoes, more than half of the respondents did not use bednets routinely and did not appreciate that the use of bednets decreases the risk of zoonotic diseases (Table 21).

Table 21: Livestock owners' awareness and experiences regarding zoonotic infections

Variables	Response	Frequency (%)	Chi-square (*P*-value)
Sex	Male	60 (68.96)	0.003 (0.580)
	Female	27 (31.03)	
Age	18–30	42 (48.28)	3.951 (0.041) *
	> 30	45 (51.72)	
Location	Amibara	47 (54.02)	0.019 (0.547)
	Haruka	40 (45.98)	
Have you ever heard of vector-borne zoonotic diseases (diseases that can be transmitted from animals to humans) affecting livestock?	Yes	54 (62.07)	2.941 (0.071)
	No	33 (37.93)	
Are you aware of the potential risks associated with consuming raw animal-origin foods (such as raw milk or undercooked meat)?	Yes	72 (82.76)	0.036 (0.578)
	No	15 (17.24)	
Have you observed any incidents of mass death among young animals in your livestock over the past years?	Yes	33 (37.93)	1.393 (0.181)
	No	54 (62.07)	
Have you noticed any evidence of abortion among your livestock over the past six months?	Yes	42 (48.28)	0.371 (0.364)
	No	45 (51.72)	
If yes, do you have a protocol for handling aborted fetuses and placental tissues to minimize disease transmission risks?	Yes	0 (0)	
	No	87 (100)	
Do you routinely use bed nets during sleep to reduce the risk of vector-borne diseases transmitted by insects (e.g. malaria or Rift Valley fever)?	Yes	24 (27.59)	1.693 (0.156)
	No	63 (72.41)	
Do you believe that using bed nets can reduce the risk of zoonotic disease transmission?	Yes	31 (35.63)	0.174 (0.448)
	No	56 (64.37)	

4.4. Knowledge, Attitude and Practices on Rift Valley Fever, West Nile Fever and other zoonotic diseases among Livestock Owners, Animal and Public health professionals

4.4.1. Sociodemographic Characteristics of the Study Population

A total of 150 (60 livestock owners, 50 public health workers and 40 para-veterinarians and animal health professionals) were interviewed during the study period using questionnaires. The mean age of participants was 36 years (range 18–74 and standard deviation 13.364) and 71.3% were male. Respondents mean duration of stay in the study area was 16.54 years (range 2-56 years, standard deviation of 11.56 years) (Table 22).

Table 22: Sociodemographic Characteristics of the KAP Study Population

Variables	Category	Frequency	Proportion (%)
Occupation	Livestock farmers	60	40
	Para-veterinarians and animal health professionals	40	26.7
	Public health professionals	50	33.3
District	Amibara	91	60.7
	Haruka	59	39.3
Sex	Male	107	71.3
	Female	43	28.7
Age	18-25 years	34	22.7
	26-35 years	63	42
	36-45ears	23	15.3
	≥46 years	30	20
Place of birth	Urban	25	16.7
	Rural	125	83.3
Educational status	Illiterate	37	24.7
	Primary school	23	15.3
	Secondary and above	90	60
Duration of stay in the study area	2-10 years	79	52.7
	11-20	16	10.7
	21-30	38	25.3
	31-40	13	8.7
	≥41	4	2.7

4.4.2. Participant's Knowledge on Rift valley fever, west Nile fever and other Zoonotic Diseases

The study result (Table 23) revealed that out of the 150 participants, 122 (81.3%) had heard the concept of 'zoonoses 'and a 91.3% KAP score was scored about their knowledge of the presence of unknown febrile infections in the study area which could be rift valley, west Nile or other zoonotic diseases.

Table 23: Participant's Knowledge on Rift valley fever, west Nile fever and other Zoonotic Diseases

Variables	Response	Frequency (n=150)	Proportion (%)	KAP score (%)
Have you ever heard/saw mass abortions (loss of pregnancy), and a high rate of severe illness and death, particularly among young animals in this area?	Yes	113	75.3	
	No	37	24.6	
Have you ever heard of, or do you know Zoonotic Diseases?	Yes	122	81.3	81.3
	No	28	18.7	
What do you think about the cause of Zoonotic Diseases	Microorganisms (virus, bacteria, parasite, fungus)	66	44	44
	Non-microorganisms	27	18	
	I don't know	57	38	
How are diseases diagnosed on this site	Clinically	124	82.7	
	Microscopic/ parasitological	26	17.3	
Is febrile illness of unknown cause common in this area	Yes	137	91.3	91.3
	No	10	6.7	
	Rarely	3	2	
Are you aware/ have you heard about mosquitoes borne viral disease such as rift valley fever and west Nile fever?	Yes	74	49.3	49.3
	No	76	50.7	
Do you know some common signs/symptoms of zoonotic diseases	Yes	76	50.7	50.7
	No	74	49.3	
Do you think that rift valley fever, west Nile fever and other zoonotic vector borne diseases are fatal/killer disease?	Yes	33	22	22
	No	36	24	
	Not sure	81	54	
Do you think that rift valley fever and west Nile fever are affecting all age groups?	Yes	28	18.7	18.7
	No	42	27	

	Not sure	80	53.3	
Do you have information on how rift valley fever, west Nile fever and other zoonotic diseases transmit?	Yes	55	36.7	36.7
	No	95	63.3	
Transmission ways of RVF, WNF and other Zoonotic Diseases?	Direct contact with infected animals and animal byproducts	32	21.3	
	Direct contact with infected materials	16	10.3	
	Consumption of raw animal origin foods	24	16	
	Inhalation	8	5.3	
	Through mosquito bite	22	14.7	
	Through sharing same shelter	14	9.3	
	Do not know	34	22.7	
What is the biting time for the mosquitoes that transmit the diseases?	Day time at home	10	6.7	
	Day time in the vegetation area	9	6	
	Nighttime at home	67	44.7	
	Both day and night	35	23.3	
	I don't know	29	19.3	
Average KAP score on RVF, WNF and others knowledge of participants (343.3507/8)				42.92%

4.4.3. Participant's attitude on Rift valley fever, west Nile fever and other Zoonotic Diseases

Only 29.3% (44/150) of the respondents perceived those Zoonotic diseases can transmit through consumption of food of animal origin or mosquito bite (Table 24). More than half 63.3% (95/150) of the respondents had no feeling of insecurity about being attacked by Rift Valley fever, West Nile fever or other zoonotic diseases. Amongst the respondents, 47.3% (71/150) of them were not sure that rift valley fever, west Nile fever viruses and other zoonotic diseases are a fatal public and animal health problem in the area. 37.3% (56/150) of the respondents perceived that it is difficult to treat Rift Valley or west Nile fever and other zoonotic diseases. 83.3% (125/150) of the respondent believed that stagnant/laying water bodies found their surroundings are a breeding site for mosquitos.

Table 24: Participant's attitude on Rift valley fever, west Nile fever and other Zoonotic Diseases

Parameter	Response	Frequency (n=150)	Proportion (%)	KAP score (%)
Do you think Zoonotic diseases Transmission through consumption raw food of animal origin or mosquito bite?	Yes	44	29.3	29.3
	No	36	24	
	I don't know	70	46.7	
Do you or members of the family feel insecure of being attacked by Rift Valley fever, West Nile fever or other zoonotic diseases after mosquito bite?	Yes	16	10.7	10.7
	No	95	63.3	
	I am not sure	39	26	
Do you agree that rift valley fever, west Nile fever viruses and other zoonotic diseases are a fatal public and animal health problem?	Yes	51	34	34
	No	28	18.7	
	I am not sure	71	47.3	
Is it simple to treat Rift Valley or west Nile fever and other zoonotic diseases?	Yes	16	10.7	10.7
	No	56	37.3	
	I am not sure	78	52	
Do you think that stagnant/laying water bodies are a breeding site for mosquitos?	Yes	125	83.3	83.3
	No	25	16.7	
If you would not go to the health facility, what is your reason?	Not sure where to go	10	6.7	
	Cost	11	7.3	
	Difficulties with transportation/distance to clinic	73	48.7	
	Do not trust medical workers	28	18.7	
	Cannot leave work	11	7.3	
	Do not want to find out that something is wrong	17	11.3	
Average KAP score on RVF, WNF and others attitude of participants (168/5)				33.6%

4.4.4. Participant's practice on Rift valley fever, west Nile fever and other Zoonotic Diseases

The study participants demonstrated a good practice, with 35.3% (53 out of 150) visiting a health center or veterinary clinic when they suspected infection by Rift Valley fever, West Nile fever, or other zoonotic diseases. However, an unacceptable practice was observed in 24.7% (37 out of 150) of participants who opted for self-treatment at home (Bad practice) instead of seeking professional care.

The result revealed that most of the respondents participated in the use of bed net and Community Education/Awareness creation and the two are the main prevention methods for rift valley fever, west Nile fever and other Zoonotic Diseases in the study area and would be considered as good practice (Table 25).

Table 25: Participant's practice on Rift valley fever, west Nile fever and other Zoonotic Diseases

Variables	Response	Frequency (n=150)	Proportion (%)	KAP score (%)
Have you ever participated in the following activities without personal protective equipment?	Herding	59	39.3	39.3
	Taking care of sick animals	13	8.7	8.7
	Assisting aborting/delivery animals	14	9.3	9.3
	Abattoir work/postmortem inspection	10	6.7	6.7
	Milking/meat processing	10	6.7	6.7
	Raw Blood drinking	1	.7	.7
	Laboratory work	43	28.7	
Did you have experience of leaving with animals in the same shelter/house?	Yes	57	38	38
	No	93	62	
Did you share the same watering point with animals?	Yes	80	53.3	53.3
	No	70	46.7	
Are there movements of animals and humans from one site/locality to another within the country and out of the country?	Yes	39	26	26
	No	111	74	
Did you have a history of living in swampy/flood prone area?	Yes	150	100	
	No	0	0	
Did you prevent mosquitoes biting you or your animals ever?	Yes	118	78.7	78.7
	No	32	21.3	
Do you have any travel history to neighboring countries?	Yes	12	8	8
	No	138	92	

Have you consulted health professionals when you or your family encountered symptoms like transient fever, rigor, headache, severe muscle and joint pain, photophobia and anorexia, extensive bleeding, and others?	Yes	48	32	32
	No	102	68	
In which prevention methods have, you participated for the prevention of vector borne zoonotic disease such as rift valley fever, west Nile fever and other Zoonotic Diseases?	Community Education/Awareness creation	25	16.7	16.7
	Avoiding c*onsumption of* raw animal origin food items	12	8	8
	Avoid sharing shelters	18	12	12
	Vaccination	16	10.7	10.7
	Restriction of domestic and wild animal movement	13	8.7	8.7
	Prevent mosquito biting	18	12	12
	Uses of bed net	36	25	25
	Spray chemicals around the home	12	8	8
If your animals, you, or/and member of your families think of being infected by rift valley, west Nile fever and other zoonotic diseases, what do you do?	Go to health center/veterinary clinic	53	35.3	35.3
	Self-treatment at home	37	24.7	
	Go to traditional healer	33	22	
	Go to religious place	27	18	
Average KAP score on RVF, WNF and others practice of participants (443.8/21)				21.133%

4.4.5. Mean Knowledge, Attitude, and Practices across Socio-Demographic Characteristics

The study found that animal health workers had higher mean scores of knowledges of rift valley fever, west Nile fever and other zoonotic disease 27.29 ± 10.96 than public health professionals and livestock farmers/owners at p = 0.000. Additionally, mean scores statistically difference were shown among Illiterate, primary education, and secondary and above educational status groups (Table 26). Mean practices scores were higher 16.94± 6.3873 for Amibara districts, than Haruka districts at p=0.000.

Table 26: Mean knowledge, attitude, and practice scores across socio-demographic characteristics.

Variable	Mean knowledge score		Mean attitude score		Mean practice score	
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
Occupation						
Livestock farmers/ owner	25.99	10.366	11.84	4.415	16.98	6.269
Animal health workers	27.29	10.96	12.86	4.07	16.85	5.156
Public health professionals	27.18	10.463	12.41	4.315	16.86	6.542
p-value	**0.000**		0.163		**0.000**	
Age						
18-25 years	27.37	11.248	12.86	4.3	16.89	6.009
26-35 years	26.43	11.077	11.95	4.203	16.97	6.204
36-45 years	26.11	10.524	12.35	4.286	16.4	6.64
≥ 46 years	27.07	10.023	12.56	4.292	17.19	6.154
p-value	0.800		0.083		0.151	

Sex						
Male	26.39	10.838	12.44	4.324	16.92	6.168
Female	27.54	10.552	12.1	4.139	16.82	6.438
p-value	0.273		.141			0.730
District						
Amibara	26.09	10.556	12.34	4.396	16.94	6.387
Haruka	22.73	10.782	12.34	4.105	16.83	5.704
p-value	**0.000**		0.098		0.000	
Place of birth						
Urban	27.28	10.547	13.4	4.254	16.72	6.064
Rural	26.6	10.847	12.12	4.275	16.93	6.282
p-value	**0.008**		0.026		0.856	
Educational status						
Illiterate	26.33	10.692	8.12	4.305	16.82	6.276
Primary education	25.43	9.526	10.91	4.323	16.82	6.04
Secondary and above	27.22	11.004	12.29	4.294	16.86	6.232
p-value	**0.041**		**0.036**		0.143	
Marital status						
Married	26.12	11.22	13.05	4.118	17.22	6
Single	26.89	10.674	11.14	4.315	16.81	6.286
p-value	0.123		**0.019**			0.220

4.5. Focus group discussion and key informant interview

Focus group discussion participants reported that "*many of the available veterinary clinics and animal health posts were not providing the required functions due to the absence of*

the necessary utilities, livestock medical supplies and well-trained animal health workers". There is an urgent need to provide the necessary utilities to run the clinics and animal health posts. Given the fact that the region in general is known for its livestock population, the available number of veterinary clinics and animal health posts seems inadequate. As a result, livestock development demands the provision of inputs required for enhancing prevention and control of zoonotic infections. From the animal health professional and para-vet side, to provide pastoralists and farmers with technical support, trained professionals should be deployed throughout the districts. Community Animal Health Workers (CAHW) are responsible for immediate taking care of animal health-related issues. In this case Amibara district is in a better position than Haruka district in terms of CAHWS. One of the key informants stated that "*The study districts has given due attention to expanding irrigation schemes as it has huge potential for irrigated agriculture which favored proliferation of vectors of diseases such as Rift valley fever, West Nile fever and other Zoonotic vector borne diseases"*.

The major activity of the healthcare sector is to facilitate the basics for health-related services. The healthcare sector that includes health posts, clinics, hospitals, and health centers in the study area and the finding of this study shows there are still problems, including a lack of health professionals in the study areas. A lack of transport (ambulance service) to a health facility was a major challenge reported in some kebeles, and from one the KII complained that of problems in health facility infrastructure including beds and road access as well as shortages of medical supplies such as provision of mosquito prevention bed nets and continuous professional development programs on arboviral infections. Additionally, the Key informant interviewee stated the top 10 diseases in the study area in 2020/21 were acute fever illness (AFI), malaria (Plasmodium falciparum), pneumonia, diarrhea, acute upper respiratory infection, malaria without laboratory confirmed, typhoid, urinary tract infection, malaria confirmed by lab, and dyspepsia (or indigestion) in that order.

4.6. Results from Observation

The current research incorporated observational data concerning environmental elements like rainfall, temperature, and humidity. Participants in the study observed instances where heavy rainfall led to the overflow of the Awash River, resulting in flooding.

Increased irrigation activities through water canals in the study area have inadvertently created favorable breeding sites for mosquito species, while also attracting livestock for grazing along the canal sides. Consequently, the aftermath of cotton crops was observed may render individuals more susceptible to mosquito bites.

The present study area revealed the presence of a wildlife sanctuary where livestock are afforded opportunities to graze, potentially leading to interactions with various wild animals, including birds, and increasing the risk of infections.

Movement of livestock is frequent within the study area. Livestock are moved primarily for two reasons: to find suitable grazing land and for marketing purposes. As a result of this movement, large herd, animals come into contact with each other, increasing the likelihood of disease transmission. Due to the close contact between animals during movement, stayed overnight at the same place and infections can easily spread among them.

The research uncovered that livestock owners engage in poor behavioral practices, including handling infected animals, assisting with deliveries using bare hands, inadequate care in abattoirs, consuming raw milk, and insufficient sanitation measures. The current research demonstrated that various human activities, such as engaging in outdoor recreation, working outdoors for occupation-related tasks, and living close to mosquito breeding sites, impact the likelihood of exposure to mosquito bites. Consequently, these factors also affect the risk of infection with West Nile Virus (WNV) and Rift Valley Fever (RVF).

5. DISCUSSIONS

This Sero-epidemiological investigation shows the detection of RVF antibodies in apparently healthy livestock population and also evaluates the risk of exposure to the virus in livestock in two purposively selected woreda's of Afar region, northeastern Ethiopia. The current study indicated seroprevalence of RVF 14.3 % in cattle, 42.2 % in Goat , 21.5% in sheep, 30.97 % in camel and 0% in donkey population with the overall seroprevalence of 22 %, which is higher than previously reported 7.6 % RVF seroprevalence in cattle from Gambella region (Asebe *et al.,* 2020). The difference in the seroprevalence might be the sample size difference (higher sample size in Gambella) and the nature of the environment as well as geographical location.

The detection of RVF in asymptomatic livestock underscores the virus's silent circulation in Ethiopia. The significantly higher seroprevalence in goats (42.2%) compared to sheep (21.5%), cattle (14.3%), and camels (31.0%) may reflect species-specific factors, such as goats' browsing behavior (increasing exposure to vectors) or heightened susceptibility to RVF infection. This finding contrasts with studies in Somalia, where comparable seroprevalence patterns were not observed (Hassan-Kadle et al., 2021), highlighting regional variability in RVF epidemiology and the need for context-specific surveillance strategies.

The difference in prevalence based on sex was reported in Chad and Madagascar, where the higher seroprevalence in males was attributed to their roles as draft and breeding animals(Kainga *et al*., 2022). But the current study showed higher seroprevalence in female than male cattle. It was learned that the overall prevailing breeding management strategy preserved more female animals for reproduction purposes than male, in a breeding ratio of approximately 1 male animal to 4 or more female animals. In the study areas dominated by indigenous cattle, they cull unwanted bulls, and artificial insemination is never practiced ever as a means of breeding.

An other study in Malawi evidenced that the circulation of RVF during the inter-epidemic period from the neighboring countries leaves open the possibility that clinical RVF cases may have occurred undetected or may have been mistaken for other diseases, as such were not reported due to lack of public awareness(Kainga *et al*., 2022). The detection of IgG in the study districts could be explained by increased permanent such as water bodies as previously reported in Tanzania and Madagascar(Obaidat *et al*., 2021). Furthermore, cross-border movement could also contribute since some of the districts share boundaries with Somalia, and Djibouti where RVF was reported by (Ibrahim *et al.,* 2021). The significant risk factors for individual livestock were species and location. The seroprevalence data from this study are vital in designing effective prevention and control strategies at national and regional levels. However, it is imperative that the role of mosquitoes, humans, and wildlife in the epidemiology of RVF in Ethiopia is elucidated.

West Nile virus infection is classically considered "endemic “in Africa, especially sub-Saharan Africa, but the precise situation of the disease in Ethiopia, has not been established well until now and the results obtained made it possible to highlight the presence of IgG West Nile in livestock sera in the districts of Amibara and Haruka, afar pastoral area. Indeed, very few studies(Asebe *et al*., 2020; Endale *et al*., 2021) have taken into account the presence of the virus in Cattle population of Gambella and South Omo Ethiopia, respectively.

In this study, significant difference in seroprevalence was observed between species of the animals tested. Highest prevalence (76.1%) was noted in donkeys followed by camel (69%), cattle (52%), Goat (34.7%) and sheep (25.7%).The current overall seroprevalence of WNV was significantly higher than that of earlier research conducted in Ethiopia particularly in areas where pastoralism is the predominant mode of livelihood (Asebe *et al.,*2020 and Endale *et al.,* 2021)and can have significant implications for both animal (Himeidan *et al*., 2014) and human health(Llorente *et al*., 2023).The reason for this relatively high seroprevalence among animals in the study area could be due to conducive nature of mosquitoes breeding site, samples were taken in the near proximity of Awash

River basin causes frequent flooding and its geographical location on the routes of migratory birds that play an important role in the epidemiology of the virus.

The seroprevalence result approved that the domestic livestock implicate as useful sentinels for WNV, although the biological basis for this remains unknown (Selim & Abdelhady, 2020; Selim *et al*., 2021). These findings may be attributed to the domestic animals present in large herds which attract a greater number of mosquitoes which is supported by Ulloa *et al*. (2009). It is interesting to note that WNV antibodies were detected in most of the domestic animals in the study districts could play a role in the maintenance and circulation of the virus among animals.

From the present study higher 70/92 (76.1%) seroprevalence of IgG to WNV infection was documented in the donkey population which significantly higher than studies from Nigeria(Idoko *et al*., 2021), Namibia(Molini *et al*., 2021), by Hassanien *et al*. (2023) Egypt in Africa; Palestine and Israel(Azmi *et al*., 2017), in turkey(Mehmet *et al*., 2017) and Spain(Gangoso *et al*., 2020). But, the current prevalence of WNV infection is slightly less than findings from Sudan 88.75%(Wegdan *et al*.) and Senegal 86.2%(Davoust *et al*., 2016).

In this study, 69% seropositivity of WNF IgG was detected in Camel which is considered as high compared with other studies like in Nigeria (17.7%) (Baba *et al*., 2014), Egypt (40%) (Selim & Abdelhady, 2020), Palestine (40%) (Azmi *et al*., 2017)and Turkey (44%)(Erol *et al*., 2016).

Previous studies showed that 4.8%(Endale *et al*., 2021) and 5.5% (Asebe *et al*., 2020) seroprevalence of IgG to WNV infection in cattle from South Omo and Gambella region, respectively. In comparison of the above findings the present study revealed significantly higher (52%) seroprevalence of WNV IgG was recorded and might be due to geographical difference and vector activity. Similar study showed 22% seroprevalence in Egypt(Selim & Abdelhady, 2020), 32.53% in Malesia(Mohammed *et al*., 2023), 20% in Turkey(Erol *et al.,* 2016). The difference between seroprevalence rates of WNV that were reported in this study and those that were reported in the earlier study might also be due in part to different sampling strategies.

Slightly higher (48.27%) seroprevalence of West Nile fever in Goat was documented in Malaysia (Mohammed *et al.,* 2023). But the present study revealed that the seroprevalence of IgG to WNV in Goat is higher (34.7%) than the seroprevalence in goats studied in Egypt 5.3% (Selim & Abdelhady, 2020)and Senegal 6.9%(Davoust *et al.,* 2016).

The current work investigated the serological prevalence of antibodies against WNV in sheep based on cELISA test was 25.7% relatively lower than other domestic animals but this result is higher than studies from Senegal 0%(Davoust *et al*., 2016), Egypt 3.5%(Selim & Abdelhady, 2020), Nigeria 20%(Olaleye *et al*., 1990), and Turkey 0%(Erol *et al*., 2016).

Multiple factors impact the transmission and distribution of WNV. Among drivers, weather conditions have direct and indirect influences on vector competence, on the vector population dynamic and on the virus replication rate within the mosquito and the large flock of sheep in the study area. Temperature plays an important role in viral replication rates and transmission of WNV. In this study, the determining factors that were found to be significantly associated with WNV exposure in the multivariable analysis were the species difference in the study area agreed with study by Paz (2015)in global context.

Factors such as sex, age, species, and districts were considered as a predicting risk factor for the seroprevalence of West Nile fever virus and Higher (51.2%) WNV seroprevalence was observed in female animals and lower (50%) WNV seroprevalence was found in old, aged animals and the seroprevalence rate is slightly higher (51.3%) in Amibara district than Haruka district. The distribution of WNV positivity was associated with district and species ($p=0.000$) but the final model of logistic regression indicated that only species was significant risk factor for seropositivity to WNV infection in the present study in the two districts.

Mosquito-borne diseases are a serious health risk in East African nations like Ethiopia (Kemunto *et al*., 2018) due to environmental conditions that allow vector-borne illnesses like malaria, rift valley fever, west Nile fever, chikungunya, and others to spread quickly. Sociodemographic characteristics and the population's KAP are important in the prevention and management of such life-threatening illnesses(Mora *et al*., 2022). Our study sought to

evaluate the knowledge, attitudes, and practices of public health specialists, animal health professionals, and owners of livestock regarding vector-borne viruses.

Veterinarians and experts in human health play a critical role in reducing the spread of zoonotic diseases to humans and animals. Notifying public health officials of zoonoses is a critical first step to safeguard the health of both humans and animals. Veterinarians and their employees may be more vulnerable to zoonotic infections due to encounters with infected animals. We investigated the beliefs, behaviors, and knowledge of public health experts and veterinarians with relation to zoonotic disease prevention strategies such wearing personal protective equipment (PPE) and the necessity of focused outreach and education indicated by Venkat *et al*. (2019).Furthermore, this study found that the frontline health workers' knowledge on rift valley, west Nile fever and other zoonotic diseases was moderate and the study is in disagreement with Mligo *et al*. (2022) in Tanzania.

Overall, the respondents exhibited a reasonable knowledge of other zoonotic diseases such as tuberculosis and brucellosis, but more than fifty percent of respondents were unable to name a single mosquito-borne disease such as Rift valley fever and West Nile fever. This study showed that overall knowledge on RVF is not associated with some socio-demographic variables like age, sex and marital status and the result was in agreement with study by Abdi *et al*. (2015)in Keniya. Education level, district and place of birth of the respondents was found to have significant positive influence on knowledge about rift valley fever, west Nile fever and other mosquito borne diseases as well as on control measures and this finding was supported by Issae *et al*. (2023)from Tanzania.

The general application of caution to prevent insect bites, study participants still indicate chemical control measures, particularly spray, as the most commonly used method by Nava-Doctor *et al*. (2021) but, in the present study area although the respondents were aware of different cultural and physical control measures, such as uses of smoking, the use of mosquito nets and covering up the skin were known to avoid mosquito bite.

The study revealed that moderate level of knowledge regarding zoonotic diseases, both among the livestock owners and among medical practitioners, and limited access to treatments or post exposure prophylaxis, constitute favorable conditions for zoonotic pathogen transmission in the case of livestock owners and the finding was in agreement with Kiffner *et al*. (2019)in northern Tanzania. Previous Study in Colombia by Jaramillo Ramírez and Álvarez (2017)showed more (26.67%) number of respondents did not know the mosquito feeding time compared with the present study 19.3% (35/150).

Educational status, occupation, district, marital status, and place of birth were among the important demographic factors that could contribute to the knowledge, attitude, and practices of animal health and public health professionals and farmers. Education opens the way for awareness and fosters a better understanding of conditions and topics in the livestock livelihood-based community. The positive influence of education was noted based on the mean score of secondary education and above, as was observed in other studies (Kainga *et al*., 2022). The mean scores for attitudes of married occupational groups were higher compared to singles most probably due to livestock management was provided by family members and similar finding in western Ethiopia(Tamiru *et al*., 2022).

The moderate knowledge among occupation differences of the role of mosquitoes in the transmission of the disease draws particular concern, as only 14.7 % of the participants knew that the mosquito is the primary vector for transmission of RVF in livestock. Whereas 22.7% of the respondents did not know that means of transmission of RFV, WNF and other zoonotic diseases and the result was in agreement with Whiteman *et al*. (2018)in Panama.

Farmers who were located further away from health facilities had poor management skills of ADs as compared to their counterparts who had better access to medical facilities. An extra minute spent walking to the health facility to seek treatment reduced the intensity of a livestock farmer's management skills of RVF by six percent. This suggested that as distance increased, the likelihood of the household members visiting health facilities declined and thus they were less likely to manage the diseases. Health facilities are the principal point for sourcing health information in many rural settings through the

distribution of education materials on signs and symptoms and prevention methods of a diseases and the find was supported by Çakmur *et al*. (2015)and Nyangau *et al*. (2021)in Turky and Keniya.

Attitude replies clearly do not correspond to practices, because many farmers admitted that despite knowing the proper action, they do not implement it either due to cost or to handwork required. This is further comprehensible considering the high risky behaviors uncovered through self-reported practices. Furthermore, the findings have weak external validity (generalizability) because of convenience and local sampling. Nevertheless, the Cronbach's alpha results, suggest that their questionnaire demonstrated a good internal consistency(Moutos *et al*., 2022).

The observed attitudes of participants could be considered a mediator between knowledge and practices and have a significant role in directing the choice of management practice. The poor attitudes of respondents regarding RVF, WNF and other zoonotic diseases were observed in the failure to associate the increased mosquito population, heavy rainfall, and flooding with increased abortions after rainy season.

Practicing high-risk behaviors such as consumption of raw milk, touching dead animals and assisting animals during parturition reveals low knowledge level of zoonotic diseases among small holder livestock owners(Özlü *et al*., 2020). The present study showed that study participants practice minimum preventive measures against zoonotic diseases risk factors. This malpractice is common in areas where farmers have low knowledge of RVF was indicated byNiraula *et al*. (2020). Although not satisfactory, the participants had a reasonable management practice score.

The average KAP score (21.133%) showed that the participants had the potential to improve with enhanced awareness. Most of the management practices for RVF are similar with respect to production practices and the prevention of other diseases. It has been observed that livestock owners in participating districts allowed livestock to graze in communal grazing grounds with mixed-livestock species. Communal grazing and mixed

species grazing accounted for the intensive interaction of livestock herds between different villages. These interactions could potentially influence the spread of RVF, WNV and other zoonotic diseases between livestock and possible spillover into humans. The risk of exposure to RVF and infection is related to their activities and to the environment. Activities involving animals increase the risk of infection due to the close contact between human beings and infected animals, which is the main transmission route of RVF to humans. Thus, people working in slaughterhouses, farmers, people living in livestock raising areas, laboratory workers and veterinarians constitute a population at risk (Tinto *et al*., 2023).

Both viruses are primarily transmitted through the bite of infected mosquitoes, particularly those of the Aedes and Culex species (Marchi *et al*., 2018). Mosquitoes become infected by feeding on the blood of infected animals and humans (Ndengu *et al*., 2021; Tajudeen *et al*., 2022).

Participants in the study observed instances where heavy rainfall led to the overflow of the Awash River, resulting in flooding. The present finding is in agreement with other studies on environmental factors such as rainfall, temperature Marchi *et al*.(2018), and humidity play a crucial role in the transmission dynamics of RVF(Garrigos *et al*., 2024). Heavy rainfall followed by periods of stagnant water provides ideal breeding grounds for mosquitoes, leading to increased vector populations and the potential for RVF outbreaks(Pham-Thanh, 2022; Shartova *et al*., 2022).

Increased irrigation activities through water canals in the study area have inadvertently created favorable breeding sites for mosquito species, while also attracting livestock for grazing along the canal sides. Consequently, the aftermath of cotton crops may render individuals more exposed to mosquito bites. Similar witness was indicated on ecological factors such as agricultural practices can alter ecosystems, leading to changes in the distribution and abundance of mosquito vectors and susceptible animal hosts. These changes can create new opportunities for RVF and WNV transmission (Jaleta *et al*., 2022; Long, 2022).

The present study area revealed the presence of a wildlife sanctuary where livestock are afforded opportunities to graze, potentially leading to interactions with various wild animals, including birds, and increasing the risk of infections. Wildlife species also serve as reservoirs for the viruses, contributing to its maintenance and spread in nature(Garrigós *et al*., 2023; Marchi *et al*., 2018). Birds are the primary reservoir hosts for the West Nile virus. The composition and density of bird populations in an area influence the amplification and maintenance of WNF virus. Certain bird species, such as corvids (e.g., crows, ravens) and passerines (e.g., sparrows, finches), are particularly susceptible to WNV infection and can serve as indicators of virus activity(Athanasakopoulou *et al*., 2023; Barbachano-Guerrero *et al*., 2019; Bergmann *et al*., 2023).

Movement of infected animals can facilitate the spread of RVF to new regions or new areas. Trade in livestock and movement of animals during seasonal migrations can contribute to the dissemination of the virus. Infected animals or mosquitoes can be transported to new areas, potentially introducing the virus to susceptible populations (Dar *et al*., 2013)

The research uncovered that livestock owners engage in poor behavioral practices, including handling infected animals, assisting with deliveries using bare hands, inadequate care in abattoirs, consuming raw milk, and insufficient sanitation measures. The current research demonstrated that various human activities, such as engaging in outdoor recreation, working outdoors for occupation-related tasks, and living close to mosquito breeding sites, impact the likelihood of exposure to mosquito bites. Consequently, these factors also affect the risk of infection with West Nile Virus (WNV) and Rift Valley Fever (RVF).

Human behaviors such as animal husbandry practices, handling of infected animals, and inadequate sanitation can increase the risk of RVF and WNV transmission to humans(Desta, 2016; Himeidan, 2016). Socioeconomic factors such as poverty, limited access to healthcare, and inadequate surveillance systems can also exacerbate the impact

of RVF and WNV outbreaks(Marchi *et al*., 2018;Houoiten *et al*., 2021). Human activities, such as outdoor recreation, occupation-related outdoor work, and residential proximity to mosquito breeding sites, influence exposure to mosquito bites and, consequently, the risk of WNV infection(Gangoso *et al*., 2020).

5.1. Limitations of the Study

The research protocol lacks funding and security concerns prevent the collection of the 1222 serum samples from humans and livestock that were intended. Furthermore, financial constraints prevented it from screening all serum samples for RVF and WNV. Lastly, it was unable to perform IgM ELISA screening, which would have assisted in differentiating between a recent viral infection and a more distant one. Another drawback of this research is that the RVF and WNV studies were limited to livestock populations; human and Bird samples were not included because ELISA kits were not available, which would have allowed for a comparison of the virus distributions among the potential hosts.

The study was not conducted using PCR to detect the causative RVF and WNV from the infected animals for confirmation the occurrence of RVF and WNF in Ethiopia and further characterized the genomic diversity of the circulating viruses in livestock of the study area.

6. CONCLUSION AND RECOMMENDATIONS

This study highlights the substantial zoonotic threat posed by Rift Valley fever (RVF) and West Nile virus (WNV) in Ethiopia's Afar region, where high seroprevalence (22.0% RVF, 50.7% WNV) and co-circulation (9.1%) in livestock underscore the urgent need for integrated surveillance and early warning systems.

In this study, significant difference in seroprevalence was observed between species of the animals tested. This study demonstrates the relatively higher seroprevalence of RVF and WNV infections in livestock animals in the Afar pastoral area of Ethiopia than previous studies in Somali region, Gambella Region and South Omo area.

The afar region is particularly vulnerable due to climatic conditions, animal husbandry practices, limited veterinary and public health infrastructure and favorable ecological situation for the existence of transmitting vectors. Hence, the scenario can lead to a potential outbreak of RVF and WNV can occur in the study area in the future.

Environmental factors such as heavy rainfall, flooding, and stagnant water create ideal breeding conditions for mosquito vectors, amplifying the risk of arboviral transmission. The presence of RVFV antibodies confirms active viral circulation, while the absence of national WNV surveillance data underscores gaps in preparedness.

Additionally, low community knowledge, attitudes, and practices (KAPs) regarding zoonotic diseases exacerbate the risk of outbreaks. These findings emphasize the urgent need for integrated, multisectoral strategies to mitigate arboviral threats, protect livelihoods, and safeguard public health in pastoral and agro-pastoral communities.

Therefore, the following recommendations are forwarded to different stakeholders to mitigate the spread and impact of zoonotic arboviruses like Rift valley fever and West Nile viruses in livestock and prevent transmission to humans.

1. **Strengthen Surveillance and Research**
    - Implement active national surveillance programs for RVFV, WNV, and other arboviruses, incorporating molecular diagnostics (e.g., rPCR) to confirm circulating strains and track viral evolution.
    - Conduct entomological studies to map mosquito vector distribution, breeding hotspots, and seasonal dynamics in flood-prone areas like the Awash basin.
    - Investigate the role of wildlife in RVFV/WNV transmission and assess cross-species spillover risks.
2. **Enhance Community Engagement and Education**
    - Launch targeted awareness campaigns to educate pastoralists, farmers, and healthcare workers on arboviral risks, preventive measures (e.g., avoiding stagnant water, using mosquito nets), and the importance of reporting suspected cases.
    - Integrate zoonotic disease education into local agricultural extension programs and school curricula to foster long-term behavioral change.
3. **Adopt a One Health Approach**
    - Establish interdisciplinary collaborations between veterinary, human health, and environmental sectors to harmonize surveillance, outbreak response, and resource allocation.
    - Conduct seroprevalence studies in humans, particularly high-risk groups (e.g., livestock handlers, butchers), to assess the burden of RVFV/WNV infections and inform clinical management.
4. **Improve Veterinary and Public Health Infrastructure**
    - Enforce routine monitoring of transboundary livestock movements to prevent cross-border viral spread.

- Develop vaccination strategies for livestock in RVFV-endemic areas to reduce viral reservoirs and human exposure.
- Invest in healthcare capacity (e.g., diagnostic tools, trained personnel) for early detection and management of human cases.

5. **Address Environmental and Socioeconomic Drivers**
    - Implement flood mitigation measures (e.g., drainage systems) in high-risk zones to disrupt mosquito breeding habitats.
    - Promote climate-resilient livelihoods to reduce community vulnerability to zoonotic disease shocks.
6. **Prioritize Policy and Funding**
    - Advocate for government and international funding to support arboviral research, surveillance, and control programs.
    - Develop evidence-based national guidelines for RVFV/WNV prevention, aligned with global health security frameworks.

By addressing these priorities, Ethiopia can build resilience against arboviral threats, protect pastoral economies, and advance toward Sustainable Development Goals (SDGs) related to health, poverty reduction, and climate action.

7. References

Abdi, I. H., Affognon, H. D., Wanjoya, A. K., Onyango-Ouma, W., & Sang, R. (2015). Knowledge, Attitudes and Practices (KAP) on Rift Valley Fever among Pastoralist Communities of Ijara District, North Eastern Kenya. *PLoS Negl Trop Dis*, *9*(11), e0004239. https://doi.org/10.1371/journal.pntd.0004239

Abdullahi, I. N., Emeribe, A. U., Ghamba, P. E., Omosigho, P. O., Bello, Z. M., Oderinde, B. S., Fasogbon, S. A., Olayemi, L., Daneji, I. M., Musa, M. H., Nwofe, J. O., Onukegbe, N. B., Okume, C. C., Musa, S., Gwarzo, A. M., & Ajagbe, O. O. R. (2020). Distribution pattern and prevalence of West Nile virus infection in Nigeria from 1950 to 2020: a systematic review. *Epidemiol Health*, *42*, e2020071. https://doi.org/10.4178/epih.e2020071

Adamu, A. M., Allam, L., Sackey, A. K. B., Nma, A. B., Mshelbwala, P. P., Machunga-Mambula, S., Idoko, S. I., Adikwu, A. A., Nafarnda, W. D., Garba, B. S., Owolodun, O. A., Dzikwi, A. A., Balogun, E. O., & Simon, A. Y. (2021). Risk factors for Rift Valley fever virus seropositivity in one-humped camels (Camelus dromedarius) and pastoralist knowledge and practices in Northern Nigeria. *One Health*, *13*, 100340. https://doi.org/10.1016/j.onehlt.2021.100340

Agboli, E., Zahouli, J. B. Z., Badolo, A., & Jost, H. (2021). Mosquito-Associated Viruses and Their Related Mosquitoes in West Africa. *Viruses*, *13*(5). https://doi.org/10.3390/v13050891

Albayrak, H., & Ozan, E. (2013). Seroepidemiological study of West Nile virus and Rift Valley fever virus in some of mammalian species (herbivores) in northern Turkey. *Journal of arthropod-borne diseases*, *7*(1), 90.

Alemayehu, G., Mamo, G., Desta, H., Alemu, B., & Wieland, B. (2021). Knowledge, attitude, and practices to zoonotic disease risks from livestock birth products among smallholder communities in Ethiopia. *One Health*, *12*, 100223. https://doi.org/10.1016/j.onehlt.2021.100223

Alemu, S. T., Ero, D., & Mor, S. M. (2023). One health insights into pastoralists' perceptions on zoonotic diseases in Ethiopia: perspectives from South Omo Zone of SNNP Region. *Pastoralism*, *13*(1), 13.

Alkan, C., Jurado-Cobena, E., & Ikegami, T. (2023). Advancements in Rift Valley fever vaccines: a historical overview and prospects for next generation candidates. *NPJ Vaccines*, *8*(1), 171. https://doi.org/10.1038/s41541-023-00769-w

Alomar, A. A., Campbell, L. P., Mathias, D. K., & Burkett-Cadena, N. D. (2023). Rift Valley Fever Virus: A Zoonotic Vector-Borne Pathogen Affecting Human and Livestock Health: ENY2099/IN1403, 5/2023. *Edis*, *2023*(3).

Artsob, H., Gubler, D. J., Enria, D. A., Morales, M. A., Pupo, M., Bunning, M. L., & Dudley, J. P. (2009). West Nile Virus in the New World: trends in the spread and

proliferation of West Nile Virus in the Western Hemisphere. *Zoonoses Public Health*, *56*(6-7), 357-369. https://doi.org/10.1111/j.1863-2378.2008.01207.x

Asebe, Mamo, G., Michlmayr, D., Abegaz, W. E., Endale, A., Medhin, G., Larrick, J. W., & Legesse, M. (2020). Seroprevalence of Rift Valley Fever and West Nile Fever in Cattle in Gambella Region, South West Ethiopia. *Vet Med (Auckl)*, *11*, 119-130. https://doi.org/10.2147/vmrr.S278867

Athanasakopoulou, Z., Sofia, M., Skampardonis, V., Giannakopoulos, A., Birtsas, P., Tsolakos, K., Spyrou, V., Chatzopoulos, D. C., Satra, M., Diamantopoulos, V., Mpellou, S., Galamatis, D., V, G. P., & Billinis, C. (2023). Indication of West Nile Virus (WNV) Lineage 2 Overwintering among Wild Birds in the Regions of Peloponnese and Western Greece. *Vet Sci*, *10*(11). https://doi.org/10.3390/vetsci10110661

Azmi, K., Tirosh-Levy, S., Manasrah, M., Mizrahi, R., Nasereddin, A., Al-Jawabreh, A., Ereqat, S., Abdeen, Z., Lustig, Y., Gelman, B., Schvartz, G., & Steinman, A. (2017). West Nile Virus: Seroprevalence in Animals in Palestine and Israel. *Vector Borne Zoonotic Dis*, *17*(8), 558-566. https://doi.org/10.1089/vbz.2016.2090

Baba, S. S., NNnadi, O. D., Hamman, K. D., Saidu, A., El Yuguda, A., & Oderinde, B. S. (2014). Preliminary study on the prevalence of West Nile virus antibody among horses, donkeys and camels in Borno State, Nigeria. *Journal of Applied Virology*, *3*(2), 39-45.

Barbachano-Guerrero, A., Vasquez-Aguilar, A. A., Aguirre, A. A., Norzagaray, A. A. Z., Gonzalez, E. C., Terrazas, A. L., & Faisal, J. L. A. (2019). West Nile Virus Prevalence in Wild Birds from Mexico. *J Wildl Dis*, *55*(2), 425-431. https://doi.org/10.7589/2018-03-065

Barzon, L., Pacenti, M., Franchin, E., Squarzon, L., Lavezzo, E., Cattai, M., Cusinato, R., & Palu, G. (2013). The complex epidemiological scenario of West Nile virus in Italy. *Int J Environ Res Public Health*, *10*(10), 4669-4689. https://doi.org/10.3390/ijerph10104669

Barzon, L., Pacenti, M., Sinigaglia, A., Berto, A., Trevisan, M., & Palu, G. (2015). West Nile virus infection in children. *Expert Rev Anti Infect Ther*, *13*(11), 1373-1386. https://doi.org/10.1586/14787210.2015.1083859

Behnke, R., & Kerven, C. (2011). Replacing pastoralism with irrigated agriculture in the Awash Valley, North-Eastern Ethiopia: Counting the costs. International Conference on the Future of Pastoralism,

Benjelloun, A., El Harrak, M., & Belkadi, B. (2016). West Nile Disease Epidemiology in North-West Africa: Bibliographical Review. *Transbound Emerg Dis*, *63*(6), e153-e159. https://doi.org/10.1111/tbed.12341

Bergmann, F., Schmoock-Wellhausen, M., Fast, C., Holicki, C. M., Michel, F., Wysocki, P., Sadeghi, B., Groschup, M. H., & Ziegler, U. (2023). Longitudinal Study of the

Occurrence of Usutu Virus and West Nile Virus Infections in Birds in a Zoological Garden in Northern Germany. *Pathogens*, *12*(6), 753.

Birungi, D., Aceng, F. L., Bulage, L., Nkonwa, I. H., Mirembe, B. B., Biribawa, C., Okethwangu, D., Opio, N. D., Monje, F., Muwanguzi, D., Ndumu, D. B., Aruho, R., Lumu, P., Lutwama, J., Kwesiga, B., & Ario, A. R. (2021). Sporadic Rift Valley Fever Outbreaks in Humans and Animals in Uganda, October 2017-January 2018. *J Environ Public Health*, *2021*, 8881191. https://doi.org/10.1155/2021/8881191

Bosco-Lauth, A. M., & Bowen, R. A. (2019). West Nile Virus: Veterinary Health and Vaccine Development. *J Med Entomol*, *56*(6), 1463-1466. https://doi.org/10.1093/jme/tjz125

Braack, L., Gouveia de Almeida, A. P., Cornel, A. J., Swanepoel, R., & De Jager, C. (2018). Mosquito-borne arboviruses of African origin: review of key viruses and vectors. *Parasites & vectors*, *11*, 1-26.

Brault, A. C. (2009). Changing patterns of West Nile virus transmission: altered vector competence and host susceptibility. *Vet Res*, *40*(2), 43. https://doi.org/10.1051/vetres/2009026

Bron, G. M., Strimbu, K., Cecilia, H., Lerch, A., Moore, S. M., Tran, Q., Perkins, T. A., & Ten Bosch, Q. A. (2021). Over 100 Years of Rift Valley Fever: A Patchwork of Data on Pathogen Spread and Spillover. *Pathogens*, *10*(6). https://doi.org/10.3390/pathogens10060708

Bronsvoort, B. M., Kelly, R. F., Freeman, E., Callaby, R., Bagninbom, J. M., Ndip, L., Handel, I. G., Tanya, V. N., Morgan, K. L., Ngwa, V. N., Rossi, G., Nfon, C. K., & Mazeri, S. (2022). A Cross-Sectional, Population-Based, Seroepidemiological Study of Rift Valley Fever in Cameroonian Cattle Populations. *Front Vet Sci*, *9*, 897481. https://doi.org/10.3389/fvets.2022.897481

Budasha, N. H., Gonzalez, J. P., Sebhatu, T. T., & Arnold, E. (2018). Rift Valley fever seroprevalence and abortion frequency among livestock of Kisoro district, South Western Uganda (2016): a prerequisite for zoonotic infection. *BMC Vet Res*, *14*(1), 271. https://doi.org/10.1186/s12917-018-1596-8

Byas, A. D., & Ebel, G. D. (2020). Comparative Pathology of West Nile Virus in Humans and Non-Human Animals. *Pathogens*, *9*(1). https://doi.org/10.3390/pathogens9010048

Cabre, O., Grandadam, M., Marié, J.-L., Gravier, P., Prangé, A., Santinelli, Y., Rous, V., Bourry, O., Durand, J.-P., & Tolou, H. (2006). West Nile virus in horses, sub-Saharan Africa. *Emerging infectious diseases*, *12*(12), 1958.

Çakmur, H., Akoğlu, L., Kahraman, E., & Atasever, M. (2015). Evaluation of farmers' knowledge-attitude-practice about zoonotic diseases in Kars, Turkey. *Kafkas Journal of Medical Sciences*, *5*(3), 87-93. https://doi.org/10.5505/kjms.2015.83436

Castillo-Olivares, J., & Wood, J. (2004). West Nile virus infection of horses. *Vet Res*, *35*(4), 467-483. https://doi.org/10.1051/vetres:2004022

Chengula, A. A., Mdegela, R. H., & Kasanga, C. J. (2013). Socio-economic impact of Rift Valley fever to pastoralists and agro pastoralists in Arusha, Manyara and Morogoro regions in Tanzania. *Springerplus*, *2*, 549. https://doi.org/10.1186/2193-1801-2-549

Chowers, M. (2009). West Nile Fever. *Tropical Diseases in Travelers*, 65-73.

Cleton, N. B., Reusken, C. B., Wagenaar, J. F., van der Vaart, E. E., Reimerink, J., van der Eijk, A. A., & Koopmans, M. P. (2015). Syndromic approach to arboviral diagnostics for global travelers as a basis for infectious disease surveillance. *PLoS neglected tropical diseases*, *9*(9), e0004073.

CSA. (2021). <Afar-Research-Report-setup.pdf>.

Dabbu Kumar, J., Jian, L., Rong, H., & Hua, Z. (2018). Emerging and Reemerging Human Viral Diseases. *Annals of Microbiology and Research*, *2*(1). https://doi.org/10.36959/958/567

Dar, O., McIntyre, S., Hogarth, S., & Heymann, D. (2013). Rift Valley fever and a new paradigm of research and development for zoonotic disease control. *Emerg Infect Dis*, *19*(2), 189-193. https://doi.org/10.3201/eid1902.120941

Datta, S., Jenks, J. A., Knudsen, D., Jensen, K., Inselman, W. M., Swanson, C. C., & Grovenburg, T. W. (2015). West Nile Virus and ferruginous hawks (Buteo regalis) in the northern Great Plains. *The Prairie Naturalist*, *47*, 38.

Daubney, R., & Hudson, J. (1931). Enzootic Hepatitis or Rift Valley Fever. An Un-described Virus Disease of Sheep, Cattle and Man from East Africa.

Davies, F. G. (2010). The historical and recent impact of Rift Valley fever in Africa. *Am J Trop Med Hyg*, *83*(2 Suppl), 73-74. https://doi.org/10.4269/ajtmh.2010.83s2a02

Davoust, B., Maquart, M., Roqueplo, C., Gravier, P., Sambou, M., Mediannikov, O., & Leparc-Goffart, I. (2016). Serological Survey of West Nile Virus in Domestic Animals from Northwest Senegal. *Vector Borne Zoonotic Dis*, *16*(5), 359-361. https://doi.org/10.1089/vbz.2015.1881

Dente, M. G., Riccardo, F., Van Bortel, W., Marrama, L., Mollet, T., Derrough, T., Sudre, B., Calistri, P., Nacca, G., Ranghiasci, A., Escadafal, C., Gaayeb, L., Guillot, A., Jimenez-Clavero, M. A., Manuguerra, J. C., Mikaty, G., Picard, M., Fernandez-Pinero, J., Perez-Ramirez, E.,…Declich, S. (2020). Enhancing Preparedness for Arbovirus Infections with a One Health Approach: The Development and Implementation of Multisectoral Risk Assessment Exercises. *Biomed Res Int*, *2020*, 4832360. https://doi.org/10.1155/2020/4832360

de Bronsvoort, B. M. C., Bagninbom, J. M., Ndip, L., Kelly, R. F., Handel, I., Tanya, V. N., Morgan, K. L., Ngu Ngwa, V., Mazeri, S., & Nfon, C. (2019). Comparison of Two Rift Valley Fever Serological Tests in Cameroonian Cattle Populations Using a Bayesian Latent Class Approach. *Front Vet Sci*, *6*, 258. https://doi.org/10.3389/fvets.2019.00258

Desta, A. H. (2016). Pastoralism and the issue of zoonoses in Ethiopia. *Journal of Biology, Agriculture and Healthcare*, *6*(7), 21-27.

Ebogo-Belobo, J. T., Kenmoe, S., Abanda, N. N., Bowo-Ngandji, A., Mbaga, D. S., Magoudjou-Pekam, J. N., Kame-Ngasse, G. I., Tchatchouang, S., Menkem, E. Z., Okobalemba, E. A., Noura, E. A., Meta-Djomsi, D., Maidadi-Foudi, M., Kenfack-Zanguim, J., Kenfack-Momo, R., Kengne-Nde, C., Esemu, S. N., Mbacham, W. F., Sadeuh-Mba, S. A.,…Njouom, R. (2023). Contemporary epidemiological data of Rift Valley fever virus in humans, mosquitoes and other animal species in Africa: A systematic review and meta-analysis. *Vet Med Sci*, *9*(5), 2309-2328. https://doi.org/10.1002/vms3.1238

EEA.(2021).Surveillance_prevention_and_control_of_WNV_and_Usutu_virus_infections_in_the_EU-EEA.pdf.

Endale, A., Michlmayr, D., Abegaz, W. E., Geda, B., Asebe, G., Medhin, G., Larrick, J. W., & Legesse, M. (2021). Sero-prevalence of West Nile virus and Rift Valley fever virus infections among cattle under extensive production system in South Omo area, southern Ethiopia. *Trop Anim Health Prod*, *53*(1), 92. https://doi.org/10.1007/s11250-020-02506-0

Erol, N., Gürçay, M., Kırdar, S., Ertuğrul, B., Gür, S., Koç, B., & Tan, M. (2016). A Serological Investigation of West Nile Virus Infections in Various Animal Species and Humans in Western Turkey. *Israel Journal of Veterinary Medicine*, *71*(2).

European Centre for Disease Prevention and Control. (2013). <west-nile-virus-risk-assessment-tool.pdf>.

Eybpoosh, S., Fazlalipour, M., Baniasadi, V., Pouriayevali, M. H., Sadeghi, F., Ahmadi Vasmehjani, A., Karbalaie Niya, M. H., Hewson, R., & Salehi-Vaziri, M. (2019). Epidemiology of West Nile Virus in the Eastern Mediterranean region: A systematic review. *PLoS Negl Trop Dis*, *13*(1), e0007081. https://doi.org/10.1371/journal.pntd.0007081

Fafetine, J., Neves, L., Thompson, P. N., Paweska, J. T., Rutten, V. P., & Coetzer, J. A. (2013). Serological evidence of Rift Valley fever virus circulation in sheep and goats in Zambezia Province, Mozambique. *PLoS Negl Trop Dis*, *7*(2), e2065. https://doi.org/10.1371/journal.pntd.0002065

FAO. (2009). RVF.

Fawzy, M., & Helmy, Y. A. (2019). The One Health Approach is Necessary for the Control of Rift Valley Fever Infections in Egypt: A Comprehensive Review. *Viruses*, *11*(2). https://doi.org/10.3390/v11020139

Fiacre, L., Pages, N., Albina, E., Richardson, J., Lecollinet, S., & Gonzalez, G. (2020). Molecular Determinants of West Nile Virus Virulence and Pathogenesis in Vertebrate and Invertebrate Hosts. *Int J Mol Sci*, *21*(23). https://doi.org/10.3390/ijms21239117

Fischer, C., Jo, W. K., Haage, V., Moreira-Soto, A., de Oliveira Filho, E. F., & Drexler, J. F. (2021). Challenges towards serologic diagnostics of emerging arboviruses. *Clin Microbiol Infect*, *27*(9), 1221-1229. https://doi.org/10.1016/j.cmi.2021.05.047

Gamino, V., & Hofle, U. (2013). Pathology and tissue tropism of natural West Nile virus infection in birds: a review. *Vet Res*, *44*(1), 39. https://doi.org/10.1186/1297-9716-44-39

Gangoso, L., Aragones, D., Martinez-de la Puente, J., Lucientes, J., Delacour-Estrella, S., Estrada Pena, R., Montalvo, T., Bueno-Mari, R., Bravo-Barriga, D., Frontera, E., Marques, E., Ruiz-Arrondo, I., Munoz, A., Oteo, J. A., Miranda, M. A., Barcelo, C., Arias Vazquez, M. S., Silva-Torres, M. I., Ferraguti, M.,…Figuerola, J. (2020). Determinants of the current and future distribution of the West Nile virus mosquito vector Culex pipiens in Spain. *Environ Res*, *188*, 109837. https://doi.org/10.1016/j.envres.2020.109837

Garcia-Carrasco, J. M., Munoz, A. R., Olivero, J., Segura, M., & Real, R. (2023). An African West Nile virus risk map for travellers and clinicians. *Travel Med Infect Dis*, *52*, 102529. https://doi.org/10.1016/j.tmaid.2022.102529

Garcia-Romero, C., Carrillo Bilbao, G. A., Navarro, J. C., Martin-Solano, S., & Saegerman, C. (2023). Arboviruses in Mammals in the Neotropics: A Systematic Review to Strengthen Epidemiological Monitoring Strategies and Conservation Medicine. *Viruses*, *15*(2). https://doi.org/10.3390/v15020417

Garrigos, M., Garrido, M., Morales-Yuste, M., Martinez-de la Puente, J., & Veiga, J. (2024). Survival effects of antibiotic exposure during the larval and adult stages in the West Nile virus vector Culex pipiens. *Insect Sci*, *31*(2), 542-550. https://doi.org/10.1111/1744-7917.13259

Garrigós, M., Garrido, M., Panisse, G., Veiga, J., & Martínez-de la Puente, J. (2023). Interactions between West Nile Virus and the Microbiota of Culex pipiens vectors: A literature review. *Pathogens*, *12*(11), 1287.

Go, Y. Y., Balasuriya, U. B., & Lee, C.-k. (2014). Zoonotic encephalitides caused by arboviruses: transmission and epidemiology of alphaviruses and flaviviruses. *Clinical and experimental vaccine research*, *3*(1), 58.

Golnar, A. J., Turell, M. J., LaBeaud, A. D., Kading, R. C., & Hamer, G. L. (2014). Predicting the mosquito species and vertebrate species involved in the theoretical transmission of Rift Valley fever virus in the United States. *PLoS neglected tropical diseases*, *8*(9), e3163.

Gregor, K., Michaely, L., Gutjahr, B., Rissmann, M., Keller, M., Dornbusch, S., Naccache, F., Schön, K., Jansen, S., & Heitmann, A. (2021). Rift Valley fever virus detection in susceptible hosts with special emphasis in insects. *Scientific reports*, *11*(1), 9822.

Gregor, K. M., Michaely, L. M., Gutjahr, B., Rissmann, M., Keller, M., Dornbusch, S., Naccache, F., Schon, K., Jansen, S., Heitmann, A., Konig, R., Brennan, B., Elliott, R. M., Becker, S., Eiden, M., Spitzbarth, I., Baumgartner, W., Puff, C., Ulrich, R., & Groschup, M. H. (2021). Rift Valley fever virus detection in susceptible hosts with special emphasis in insects. *Sci Rep*, *11*(1), 9822. https://doi.org/10.1038/s41598-021-89226-z

Gulati, B. R. (2014). West Nile Virus Infection among Animals and Humans in India. *Advances in Animal and Veterinary Sciences*, *2*(4S), 17-23. https://doi.org/10.14737/journal.aavs/2014/2.4s.17.23

Gutu, M. A., Bekele, A., Seid, Y., Mohammed, Y., Gemechu, F., Woyessa, A. B., Tayachew, A., Dugasa, Y., Gizachew, L., Idosa, M., Tokarz, R. E., & Sugerman, D. (2021). Another dengue fever outbreak in Eastern Ethiopia-An emerging public health threat. *PLoS Negl Trop Dis*, *15*(1), e0008992. https://doi.org/10.1371/journal.pntd.0008992

Habarugira, G., Suen, W. W., Hobson-Peters, J., Hall, R. A., & Bielefeldt-Ohmann, H. (2020). West Nile Virus: An Update on Pathobiology, Epidemiology, Diagnostics, Control and "One Health" Implications. *Pathogens*, *9*(7). https://doi.org/10.3390/pathogens9070589

Hammami, S., Hassine, T. B., Conte, A., Amdouni, J., De Massis, F., Sghaier, S., & Hassen, S. B. (2017). West Nile disease in Tunisia: an overview of 60 years. *Vet Ital*, *53*(3), 225-234. https://doi.org/10.12834/VetIt.1181.6565.2

Hartman, A. (2017). Rift Valley Fever. *Clin Lab Med*, *37*(2), 285-301. https://doi.org/10.1016/j.cll.2017.01.004

Hassan-Kadle, A. A., Osman, A. M., Shair, M. A., Abdi, O. M., Yusuf, A. A., Ibrahim, A. M., & Vieira, R. F. (2021). Rift Valley fever and Brucella spp. in ruminants, Somalia. *BMC Veterinary Research*, *17*(1), 280.

Hassan, A., Muturi, M., Mwatondo, A., Omolo, J., Bett, B., Gikundi, S., Konongoi, L., Ofula, V., Makayotto, L., Kasiti, J., Oele, E., Onyango, C., Gura, Z., Njenga, K., & Munyua, P. (2020). Epidemiological Investigation of a Rift Valley Fever Outbreak in Humans and Livestock in Kenya, 2018. *Am J Trop Med Hyg*, *103*(4), 1649-1655. https://doi.org/10.4269/ajtmh.20-0387

Hassanien, R. T., Hussein, H. A., Abdelmegeed, H. K., Abdelwahed, D. A., Khattab, O. M., Ali, M. H., Habashi, A. R., Ibraheem, E. M., Shahein, M. A., & Abohatab, E. M. (2023). West Nile virus: The current situation in Egypt. *Vet World*, *16*(5), 1154-1160. https://doi.org/10.14202/vetworld.2023.1154-1160

Heinrich, N., Saathoff, E., Weller, N., Clowes, P., Kroidl, I., Ntinginya, E., Machibya, H., Maboko, L., Loscher, T., Dobler, G., & Hoelscher, M. (2012). High seroprevalence of Rift Valley FEVER AND EVIDENCE FOR ENDEMIC circulation in Mbeya region, Tanzania, in a cross-sectional study. *PLoS Negl Trop Dis*, *6*(3), e1557. https://doi.org/10.1371/journal.pntd.0001557

Helmy, Y. A., El-Adawy, H., & Abdelwhab, E. M. (2017). A comprehensive review of common bacterial, parasitic and viral zoonoses at the human-animal interface in Egypt. *Pathogens*, *6*(3), 33.

Himeidan, Y. E. (2016). Rift Valley fever: current challenges and future prospects. *Res Rep Trop Med*, *7*, 1-9. https://doi.org/10.2147/RRTM.S63520

Himeidan, Y. E., Kweka, E. J., Mahgoub, M. M., El Rayah el, A., & Ouma, J. O. (2014). Recent outbreaks of rift valley Fever in East Africa and the middle East. *Front Public Health*, *2*, 169. https://doi.org/10.3389/fpubh.2014.00169

Holcomb, K. M., Mathis, S., Staples, J. E., Fischer, M., Barker, C. M., Beard, C. B., Nett, R. J., Keyel, A. C., Marcantonio, M., Childs, M. L., Gorris, M. E., Rochlin, I., Hamins-Puertolas, M., Ray, E. L., Uelmen, J. A., DeFelice, N., Freedman, A. S., Hollingsworth, B. D., Das, P.,…Johansson, M. A. (2023). Evaluation of an open forecasting challenge to assess skill of West Nile virus neuroinvasive disease prediction. *Parasit Vectors*, *16*(1), 11. https://doi.org/10.1186/s13071-022-05630-y

Ibrahim, M., Schelling, E., Zinsstag, J., Hattendorf, J., Andargie, E., & Tschopp, R. (2021). Sero-prevalence of brucellosis, Q-fever and Rift Valley fever in humans and livestock in Somali Region, Ethiopia. *PLoS Negl Trop Dis*, *15*(1), e0008100. https://doi.org/10.1371/journal.pntd.0008100

Idoko, I. S., Schvartz, G., Tirosh-Levy, S., Erster, O., Jibril, J. Y., Adamu, A. M., Enem, S. I., Omeje, J. N., Nafarnda, W. D., & Steinman, A. (2021). West Nile virus neutralizing antibody prevalence in donkeys from northern Nigeria. *Trans R Soc Trop Med Hyg*, *115*(5), 566-568. https://doi.org/10.1093/trstmh/traa104

Ikegami, T., & Makino, S. (2011). The pathogenesis of Rift Valley fever. *Viruses*, *3*(5), 493-519. https://doi.org/10.3390/v3050493

Issae, A. R., Katakweba, A. A. S., Kicheleri, R. P., Chengula, A. A., & Kasanga, C. J. (2023). Knowledge, attitudes and practices on rift valley fever among pastoral and agropastoral communities of Ngorongoro in the rift valley ecosystem, Tanzania, conducted in 2021/2022. *PLoS Negl Trop Dis*, *17*(8), e0011560. https://doi.org/10.1371/journal.pntd.0011560

Jaleta, M. B., Tefera, M., Negussie, H., Mulatu, T., Berhe, T., Belete, F., Yalew, B., Gizaw, O., Dabasa, G., Abunna, F., Regassa, F., Amenu, K., & Leta, S. (2022). Entomological survey of the potential vectors of Rift Valley fever virus and absence of detection of the virus genome from the vectors in various niches in the southern half of the Great Rift Valley of Ethiopia. *Vet Med Sci*, *8*(6), 2716-2725. https://doi.org/10.1002/vms3.941

Jaramillo Ramírez, G. I., & Álvarez, L. S. B. (2017). Knowledge, attitudes and practices regarding dengue, chikungunya, and Zika and their vector in Villavicencio, Colombia. *The Open Public Health Journal*, *10*(1).

Javelle, E., Lesueur, A., Pommier de Santi, V., de Laval, F., Lefebvre, T., Holweck, G., Durand, G. A., Leparc-Goffart, I., Texier, G., & Simon, F. (2020). The challenging management of Rift Valley Fever in humans: literature review of the clinical disease and algorithm proposal. *Ann Clin Microbiol Antimicrob*, *19*(1), 4. https://doi.org/10.1186/s12941-020-0346-5

Johnson, N., Voller, K., Phipps, L. P., Mansfield, K., & Fooks, A. R. (2012). Rapid molecular detection methods for arboviruses of livestock of importance to northern Europe. *BioMed Research International*, *2012*.

Juma, J., Fonseca, V., Konongoi, S. L., Heusden, P., Roesel, K., Sang, R., Bett, B., Christoffels, A., Oliveira, T., & Oyola, S. O. (2022). https://doi.org/10.21203/rs.3.rs-1415684/v1

Kainga, H., Phonera, M. C., Chatanga, E., Kallu, S. A., Mpundu, P., Samutela, M., Chambaro, H. M., Kajihara, M., Shempela, D. M., Sikalima, J., Muleya, W., Shawa, M., Chulu, J., Njunga, G., Simuunza, M., Takada, A., Sawa, H., Simulundu, E., & Saasa, N. (2022). Seroprevalence and Associated Risk Factors of Rift Valley Fever in Livestock from Three Ecological Zones of Malawi. *Pathogens*, *11*(11). https://doi.org/10.3390/pathogens11111349

Kamalia, M. A., Ayoub, M. E., Bureau, B. L., Abdel-Reheem, A.-R. K., & Jha, P. (2022). Acute Fever of Unknown Origin: A Presentation of West Nile Encephalitis. *WMJ*, *121*(2), E22-E26.

Kemunto, N., Mogoa, E., Osoro, E., Bitek, A., Kariuki Njenga, M., & Thumbi, S. M. (2018). Zoonotic disease research in East Africa. *BMC Infect Dis*, *18*(1), 545. https://doi.org/10.1186/s12879-018-3443-8

Kiffner, C., Latzer, M., Vise, R., Benson, H., Hammon, E., & Kioko, J. (2019). Comparative knowledge, attitudes, and practices regarding anthrax, brucellosis, and rabies in three districts of northern Tanzania. *BMC Public Health*, *19*(1), 1625. https://doi.org/10.1186/s12889-019-7900-0

Kilpatrick, A. M., LaDeau, S. L., & Marra, P. P. (2007). Ecology of West Nile virus transmission and its impact on birds in the western hemisphere. *The Auk*, *124*(4), 1121-1136.

Klingelhofer, D., Braun, M., Kramer, I. M., Reuss, F., Muller, R., Groneberg, D. A., & Bruggmann, D. (2023). A virus becomes a global concern: research activities on West-Nile virus. *Emerg Microbes Infect*, *12*(2), 2256424. https://doi.org/10.1080/22221751.2023.2256424

Kwasnik, M., Rozek, W., & Rola, J. (2021). Rift Valley Fever - a Growing Threat To Humans and Animals. *J Vet Res*, *65*(1), 7-14. https://doi.org/10.2478/jvetres-2021-0009

Lancelot, R., Beral, M., Rakotoharinome, V. M., Andriamandimby, S. F., Heraud, J. M., Coste, C., Apolloni, A., Squarzoni-Diaw, C., de La Rocque, S., Formenty, P. B., Bouyer, J., Wint, G. R., & Cardinale, E. (2017). Drivers of Rift Valley fever epidemics in Madagascar. *Proc Natl Acad Sci U S A*, *114*(5), 938-943. https://doi.org/10.1073/pnas.1607948114

Lanciotti, R. S., Roehrig, J. T., Deubel, V., Smith, J., Parker, M., Steele, K., Crise, B., Volpe, K. E., Crabtree, M. B., Scherret, J. H., Hall, R. A., MacKenzie, J. S., Cropp, C. B., Panigrahy, B., Ostlund, E., Schmitt, B., Malkinson, M., Banet, C., Weissman,

J.,…Gubler, D. J. (1999). Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science*, *286*(5448), 2333-2337. https://doi.org/10.1126/science.286.5448.2333

Lapa, D., Pauciullo, S., Ricci, I., Garbuglia, A. R., Maggi, F., Scicluna, M. T., & Tofani, S. (2024). Rift Valley Fever Virus: An Overview of the Current Status of Diagnostics. *Biomedicines*, *12*(3). https://doi.org/10.3390/biomedicines12030540

Leta, S., Beyene, T. J., De Clercq, E. M., Amenu, K., Kraemer, M. U. G., & Revie, C. W. (2018). Global risk mapping for major diseases transmitted by Aedes aegypti and Aedes albopictus. *Int J Infect Dis*, *67*, 25-35. https://doi.org/10.1016/j.ijid.2017.11.026

Lichoti, J. K., Kihara, A., Oriko, A. A., Okutoyi, L. A., Wauna, J. O., Tchouassi, D. P., Tigoi, C. C., Kemp, S., Sang, R., & Mbabu, R. M. (2014). Detection of rift valley Fever virus interepidemic activity in some hotspot areas of kenya by sentinel animal surveillance, 2009-2012. *Vet Med Int*, *2014*, 379010. https://doi.org/10.1155/2014/379010

Linthicum, K. J., Britch, S. C., & Anyamba, A. (2016). Rift Valley Fever: An Emerging Mosquito-Borne Disease. *Annu Rev Entomol*, *61*, 395-415. https://doi.org/10.1146/annurev-ento-010715-023819

Llorente, F., Gutierrez-Lopez, R., Perez-Ramirez, E., Sanchez-Seco, M. P., Herrero, L., Jimenez-Clavero, M. A., & Vazquez, A. (2023). Experimental infections in red-legged partridges reveal differences in host competence between West Nile and Usutu virus strains from Southern Spain. *Front Cell Infect Microbiol*, *13*, 1163467. https://doi.org/10.3389/fcimb.2023.1163467

Lvov, D. K., Shchelkanov, M. Y., Alkhovsky, S. V., & Deryabin, P. G. (2015). Single-Stranded RNA Viruses. In *Zoonotic Viruses in Northern Eurasia* (pp. 135-392). https://doi.org/10.1016/b978-0-12-801742-5.00008-8

Mada, P. K., Sneed, P., Castano, G., Moore, M., & Joel Chandranesan, A. S. (2020). Retrospective Descriptive Analysis of West Nile Neuroinvasive Disease (WNND) in Northwest Louisiana. *Int J Microbiol*, *2020*, 3513859. https://doi.org/10.1155/2020/3513859

Marchi, S., Trombetta, C. M., & Montomoli, E. (2018). Emerging and re-emerging arboviral diseases as a global health problem. *Public Health: Emerging and Re-emerging Issues. United Kindgom: IntechOpen*, 25-46.

Matiko, M. K., Salekwa, L. P., Kasanga, C. J., Kimera, S. I., Evander, M., & Nyangi, W. P. (2018). Serological evidence of inter-epizootic/inter-epidemic circulation of Rift Valley fever virus in domestic cattle in Kyela and Morogoro, Tanzania. *PLoS neglected tropical diseases*, *12*(11), e0006931.

Mayer, S. V., Tesh, R. B., & Vasilakis, N. (2017). The emergence of arthropod-borne viral diseases: A global prospective on dengue, chikungunya and zika fevers. *Acta Trop*, *166*, 155-163. https://doi.org/10.1016/j.actatropica.2016.11.020

Mehmet, K., Sibel, G., Orhan, Y., Nuri, M., Sibel, Y., Sibel, H., Oya, B., & Metin, G. (2017). Serological investigation of West Nile Virus infection in domestic horses and donkeys in Turkey.

Mekuriaw, W., Kinde, S., Kindu, B., Mulualem, Y., Hailu, G., Gebresilassie, A., Sisay, C., Bekele, F., Amare, H., Wossen, M., Woyessa, A., Cross, C. L., & Messenger, L. A. (2022). Epidemiological, Entomological, and Climatological Investigation of the 2019 Dengue Fever Outbreak in Gewane District, Afar Region, North-East Ethiopia. *Insects*, *13*(11). https://doi.org/10.3390/insects13111066

Mencattelli, G., Ndione, M. H. D., Rosa, R., Marini, G., Diagne, C. T., Diagne, M. M., Fall, G., Faye, O., Diallo, M., Faye, O., Savini, G., & Rizzoli, A. (2022). Epidemiology of West Nile virus in Africa: An underestimated threat. *PLoS Negl Trop Dis*, *16*(1), e0010075. https://doi.org/10.1371/journal.pntd.0010075

Metras, R., Edmunds, W. J., Youssouffi, C., Dommergues, L., Fournie, G., Camacho, A., Funk, S., Cardinale, E., Le Godais, G., Combo, S., Filleul, L., Youssouf, H., & Subiros, M. (2020). Estimation of Rift Valley fever virus spillover to humans during the Mayotte 2018-2019 epidemic. *Proc Natl Acad Sci U S A*, *117*(39), 24567-24574. https://doi.org/10.1073/pnas.2004468117

Mhina, A. D., Kasanga, C. J., Sindato, C., Karimuribo, E. D., & Mboera, L. E. G. (2015). Rift Valley fever potential mosquito vectors and their infection status in Ngorongoro District in northern Tanzania. *Tanzania Journal of Health Research*, *17*(4). https://doi.org/10.4314/thrb.v17i4.3

Mligo, B. J., Sindato, C., Yapi, R. B., Mathew, C., Mkupasi, E. M., Kazwala, R. R., & Karimuribo, E. D. (2022). Knowledge, attitude and practices of frontline health workers in relation to detection of brucellosis in rural settings of Tanzania: a cross-sectional study. *One Health Outlook*, *4*(1), 1. https://doi.org/10.1186/s42522-021-00056-5

Mohammed, M. N., Yasmin, A. R., Ramanoon, S. Z., Noraniza, M. A., Ooi, P. T., Ain-Najwa, M. Y., Natasha, J. A., Nur-Fazila, S. H., Arshad, S. S., & Mohammed, H. O. (2023). Serological and molecular surveillance of West Nile virus in domesticated mammals of peninsular Malaysia. *Front Vet Sci*, *10*, 1126199. https://doi.org/10.3389/fvets.2023.1126199

Molini, U., Franzo, G., Nel, H., Khaiseb, S., Ntahonshikira, C., Chiwome, B., Baines, I., Madzingira, O., Monaco, F., Savini, G., & D'Alterio, N. (2021). West Nile Virus Seroprevalence in a Selected Donkey Population of Namibia. *Front Vet Sci*, *8*, 681354. https://doi.org/10.3389/fvets.2021.681354

Mor, S. M., Wiethoelter, A. K., Massey, P., & Eastwood, K. Effective surveillance and response relies on networks linking diverse information sources (Gresham *et al*. 2013). In the context of One Health, this can include gathering disease and population statistics from humans, domestic and wild animals, coupled with data on the.

Mora, C., McKenzie, T., Gaw, I. M., Dean, J. M., von Hammerstein, H., Knudson, T. A., Setter, R. O., Smith, C. Z., Webster, K. M., Patz, J. A., & Franklin, E. C. (2022). Over half of known human pathogenic diseases can be aggravated by climate change. *Nat Clim Chang*, *12*(9), 869-875. https://doi.org/10.1038/s41558-022-01426-1

Moutos, A., Doxani, C., Stefanidis, I., Zintzaras, E., & Rachiotis, G. (2022). Knowledge, Attitude and Practices (KAP) of Ruminant Livestock Farmers Related to Zoonotic Diseases in Elassona Municipality, Greece. *Eur J Investig Health Psychol Educ*, *12*(3), 269-280. https://doi.org/10.3390/ejihpe12030019

Nava-Doctor, J. E., Sandoval-Ruiz, C. A., & Fernandez-Crispin, A. (2021). Knowledge, attitudes, and practices regarding vector-borne diseases in central Mexico. *J Ethnobiol Ethnomed*, *17*(1), 45. https://doi.org/10.1186/s13002-021-00471-y

Ndengu, M., Matope, G., Tivapasi, M., Pfukenyi, D. M., Cetre-Sossah, C., & De Garine-Wichatitsky, M. (2020). Seroprevalence and associated risk factors of Rift Valley fever in cattle and selected wildlife species at the livestock/wildlife interface areas of Gonarezhou National Park, Zimbabwe. *Onderstepoort J Vet Res*, *87*(1), e1-e7. https://doi.org/10.4102/ojvr.v87i1.1731

Ng'ang'a, C. M., Bukachi, S. A., & Bett, B. K. (2016). Lay perceptions of risk factors for Rift Valley fever in a pastoral community in northeastern Kenya. *BMC Public Health*, *16*, 32. https://doi.org/10.1186/s12889-016-2707-8

Nielsen, S. S., Alvarez, J., Bicout, D. J., Calistri, P., Depner, K., Drewe, J. A., Garin-Bastuji, B., Rojas, J. L. G., Schmidt, C. G., Michel, V., Chueca, M. A. M., Roberts, H. C., Sihvonen, L. H., Stahl, K., Calvo, A. V., Viltrop, A., Winckler, C., Bett, B., Cetre-Sossah, C.,…Zancanaro, G. (2020). Rift Valley Fever - epidemiological update and risk of introduction into Europe. *EFSA J*, *18*(3), e06041. https://doi.org/10.2903/j.efsa.2020.6041

Nin-Pratt, A., Jabbar, M. A., & Ehui, S. (2009). Benefits and costs of compliance of sanitary regulations in livestock markets: the case of Rift Valley Fever in the Somali region of Ethiopia. *Quarterly Journal of International Agriculture*, *48*(3), 219-241.

Niraula, A., Sharma, A., & Dahal, U. (2020). Knowledge, Attitude and Practices (Kap) Regarding Zoonotic Diseases among Smallholder Livestock Owners of Tulsipur Sub-Metropolitan City, Nepal. *Journal of Healthcare in Developing Countries*, *1*(3), 41-46. https://doi.org/10.26480/jhcdc.03.2021.41.46

Nyakarahuka, L., de St. Maurice, A., Purpura, L., Ervin, E., Balinandi, S., Tumusiime, A., Kyondo, J., Mulei, S., Tusiime, P., & Lutwama, J. (2018). Prevalence and risk factors of Rift Valley fever in humans and animals from Kabale district in Southwestern Uganda, 2016. *PLoS neglected tropical diseases*, *12*(5), e0006412.

Nyangau, P. N., Nzuma, J. M., Irungu, P., & Kassie, M. (2021). Evaluating livestock farmers knowledge, beliefs, and management of arboviral diseases in Kenya: A

multivariate fractional probit approach. *PLoS Negl Trop Dis*, *15*(9), e0009786. https://doi.org/10.1371/journal.pntd.0009786

Obaidat, M. M., Graziano, J. C., Morales-Betoulle, M., Brown, S. M., Chiang, C.-F., & Klena, J. D. (2021). Rift Valley fever and Crimean-Congo hemorrhagic fever viruses in ruminants, Jordan. *Emerging infectious diseases*, *27*(2), 653.

OIE. (2018). <rift-valley-fever --laboratory manual.pdf>.

Olaleye, O. D., Omilabu, S. A., Ilomechina, E. N., & Fagbami, A. H. (1990). A survey for haemagglutination-inhibiting antibody to West Nile virus in human and animal sera in Nigeria. *Comp Immunol Microbiol Infect Dis*, *13*(1), 35-39. https://doi.org/10.1016/0147-9571(90)90006-f

Olive, M. M., Chevalier, V., Grosbois, V., Tran, A., Andriamandimby, S. F., Durand, B., Ravalohery, J. P., Andriamamonjy, S., Rakotomanana, F., Rogier, C., & Heraud, J. M. (2016). Integrated Analysis of Environment, Cattle and Human Serological Data: Risks and Mechanisms of Transmission of Rift Valley Fever in Madagascar. *PLoS Negl Trop Dis*, *10*(7), e0004827. https://doi.org/10.1371/journal.pntd.0004827

Oluwayelu, D., Adebiyi, A., & Tomori, O. (2018). Endemic and emerging arboviral diseases of livestock in Nigeria: a review. *Parasit Vectors*, *11*(1), 337. https://doi.org/10.1186/s13071-018-2911-8

Ozcelik, R., Abakar, M. F., Counotte, M. J., Abdelrazak Zakaria, F., Kimala, P., Issa, R., & Durr, S. (2023). Seroprevalence and associated risk factors of brucellosis, Rift Valley fever and Q fever among settled and mobile agro-pastoralist communities and their livestock in Chad. *PLoS Negl Trop Dis*, *17*(6), e0011395. https://doi.org/10.1371/journal.pntd.0011395

Özlü, H., Atasever, M., & Atasever, M. A. (2020). Knowledge, attitude, and practices of cattle farmers regarding zoonotic diseases in Erzurum, Turkey. *Austral journal of veterinary sciences*, *52*(3), 79-85.

Paweska, J. (2008). Epidemiology of RVF: Potential Risks for introduction into Europe. Workshop on Symposium on Emerging Vector Born Viral Disease Lelystad, Netherlands,

Pawęska, J. T., Jansen van Vuren, P., Msimang, V., Lô, M. M., Thiongane, Y., Mulumba-Mfumu, L. K., Mansoor, A., Fafetine, J. M., Magona, J. W., & Boussini, H. (2021). Large-Scale International Validation of an Indirect ELISA Based on Recombinant Nucleocapsid Protein of Rift Valley Fever Virus for the Detection of IgG Antibody in Domestic Ruminants. *Viruses*, *13*(8), 1651.

Paz, S. (2015). Climate change impacts on West Nile virus transmission in a global context. *Philos Trans R Soc Lond B Biol Sci*, *370*(1665). https://doi.org/10.1098/rstb.2013.0561

Pepin, M., Bouloy, M., Bird, B. H., Kemp, A., & Paweska, J. (2010). Rift Valley fever virus(Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular

epidemiology, vectors, diagnostics and prevention. *Vet Res*, *41*(6), 61. https://doi.org/10.1051/vetres/2010033

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Karp, N. A., Lazic, S. E., Lidster, K., MacCallum, C. J., Macleod, M.,…Wurbel, H. (2020). The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *BMJ Open Sci*, *4*(1), e100115. https://doi.org/10.1136/bmjos-2020-100115

Pérez-Ramírez, E., Cano-Gómez, C., Llorente, F., Adzic, B., Al Ameer, M., Djadjovski, I., El Hage, J., El Mellouli, F., Goletic, T., & Hovsepyan, H. (2020). External quality assessment of Rift Valley fever diagnosis in 17 veterinary laboratories of the Mediterranean and Black Sea regions. *PLoS One*, *15*(9), e0239478.

Petersen *et al*. (2013). West Nile Virus, a Reemerging Virus. *Precision Medicine*. https://doi.org/10.14800/pm.604

Peyre, M., Chevalier, V., Abdo-Salem, S., Velthuis, A., Antoine-Moussiaux, N., Thiry, E., & Roger, F. (2015). A Systematic Scoping Study of the Socio-Economic Impact of Rift Valley Fever: Research Gaps and Needs. *Zoonoses Public Health*, *62*(5), 309-325. https://doi.org/10.1111/zph.12153

Pham-Thanh, L. (2022). *The influence of urban livestock-keeping on the epidemiology of mosquito-borne zoonotic flaviviruses in Hanoi city of Vietnam* Acta Universitatis Upsaliensis].

Poueme Namegni, R. S., Njan-Nloga, A. M., Wade, A., Eisenbarth, A., Groschup, M. H., & Stoek, F. (2020). Diversity and abundance of potential vectors of rift valley fever virus in the north region of Cameroon. *Insects*, *11*(11), 814.

Reisen, W. K. (2013). Ecology of West Nile virus in North America. *Viruses*, *5*(9), 2079-2105. https://doi.org/10.3390/v5092079

Rimoldi, G., Mete, A., Adaska, J. M., Anderson, M. L., Symmes, K. P., & Diab, S. (2017). West Nile Virus Infection in Sheep. *Vet Pathol*, *54*(1), 155-158. https://doi.org/10.1177/0300985816653796

Ringot, D., Durand, J. P., Toulou, H., Boutin, J. P., & Davoust, B. (2004). Rift Valley fever in Chad. *Emerg Infect Dis*, *10*(5), 945-947. https://doi.org/10.3201/eid1005.030621

Roger, M., Beral, M., Licciardi, S., Soule, M., Faharoudine, A., Foray, C., Olive, M. M., Maquart, M., Soulaimane, A., Madi Kassim, A., Cetre-Sossah, C., & Cardinale, E. (2014). Evidence for circulation of the rift valley fever virus among livestock in the union of Comoros. *PLoS Negl Trop Dis*, *8*(7), e3045. https://doi.org/10.1371/journal.pntd.0003045

Rossi, S. L., Ross, T. M., & Evans, J. D. (2010). West Nile virus. *Clin Lab Med*, *30*(1), 47-65. https://doi.org/10.1016/j.cll.2009.10.006

Sadeuh-Mba, S. A., Yonga Wansi, G. M., Demanou, M., Gessain, A., & Njouom, R. (2018). Serological evidence of rift valley fever Phlebovirus and Crimean-Congo

hemorrhagic fever orthonairovirus infections among pygmies in the east region of Cameroon. *Virology journal*, *15*, 1-5.

Sado, F. Y., Tchetgna, H. S., Kamgang, B., Djonabaye, D., Nakoune, E., McCall, P. J., Ndip, R. N., & Wondji, C. S. (2022). Seroprevalence of Rift Valley fever virus in domestic ruminants of various origins in two markets of Yaounde, Cameroon. *PLoS Negl Trop Dis*, *16*(8), e0010683. https://doi.org/10.1371/journal.pntd.0010683

Salekwa, L. P., Wambura, P. N., Matiko, M. K., & Watts, D. M. (2019). Circulation of Rift Valley Fever Virus Antibody in Cattle during Inter-Epizootic/Epidemic Periods in Selected Regions of Tanzania. *Am J Trop Med Hyg*, *101*(2), 459-466. https://doi.org/10.4269/ajtmh.18-0798

Sambri, V., Capobianchi, M., Charrel, R., Fyodorova, M., Gaibani, P., Gould, E., Niedrig, M., Papa, A., Pierro, A., Rossini, G., Varani, S., Vocale, C., & Landini, M. P. (2013). West Nile virus in Europe: emergence, epidemiology, diagnosis, treatment, and prevention. *Clin Microbiol Infect*, *19*(8), 699-704. https://doi.org/10.1111/1469-0691.12211

Sang, R., Arum, S., Chepkorir, E., Mosomtai, G., Tigoi, C., Sigei, F., Lwande, O. W., Landmann, T., Affognon, H., & Ahlm, C. (2017). Distribution and abundance of key vectors of Rift Valley fever and other arboviruses in two ecologically distinct counties in Kenya. *PLoS neglected tropical diseases*, *11*(2), e0005341.

Sang, R., Kioko, E., Lutomiah, J., Warigia, M., Ochieng, C., O'Guinn, M., Lee, J. S., Koka, H., Godsey, M., Hoel, D., Hanafi, H., Miller, B., Schnabel, D., Breiman, R. F., & Richardson, J. (2010). Rift Valley fever virus epidemic in Kenya, 2006/2007: the entomologic investigations. *Am J Trop Med Hyg*, *83*(2 Suppl), 28-37. https://doi.org/10.4269/ajtmh.2010.09-0319

Sang, R., Lutomiah, J., Said, M., Makio, A., Koka, H., Koskei, E., Nyunja, A., Owaka, S., Matoke-Muhia, D., & Bukachi, S. (2017). Effects of irrigation and rainfall on the population dynamics of Rift Valley fever and other arbovirus mosquito vectors in the epidemic-prone Tana River County, Kenya. *Journal of medical entomology*, *54*(2), 460-470.

Santos, P. D., Michel, F., Wylezich, C., Hoper, D., Keller, M., Holicki, C. M., Szentiks, C. A., Eiden, M., Muluneh, A., Neubauer-Juric, A., Thalheim, S., Globig, A., Beer, M., Groschup, M. H., & Ziegler, U. (2022). Co-infections: Simultaneous detections of West Nile virus and Usutu virus in birds from Germany. *Transbound Emerg Dis*, *69*(2), 776-792. https://doi.org/10.1111/tbed.14050

Sargeant, J. M., & O'Connor, A. M. (2020). Scoping Reviews, Systematic Reviews, and Meta-Analysis: Applications in Veterinary Medicine. *Front Vet Sci*, *7*, 11. https://doi.org/10.3389/fvets.2020.00011

Sayed-Ahmed, M. (2016). Incidence History of West Nile Virus in Africa and Middle East, With an Emphasis on Egypt: A Review. *Journal of Dairy, Veterinary & Animal Research*, *3*(3). https://doi.org/10.15406/jdvar.2016.03.00080

Selim, A., & Abdelhady, A. (2020). The first detection of anti-West Nile virus antibody in domestic ruminants in Egypt. *Trop Anim Health Prod*, *52*(6), 3147-3151. https://doi.org/10.1007/s11250-020-02339-x

Selim, A., & Abdelhady, A. (2020). The first detection of anti-West Nile virus antibody in domestic ruminants in Egypt. *Tropical Animal Health and Production*, *52*(6), 3147-3151.

Selim, A., Megahed, A., Kandeel, S., Alouffi, A., & Almutairi, M. M. (2021). West Nile virus seroprevalence and associated risk factors among horses in Egypt. *Sci Rep*, *11*(1), 20932. https://doi.org/10.1038/s41598-021-00449-6

Seufi, A. M., & Galal, F. H. (2010). Role of Culex and Anopheles mosquito species as potential vectors of rift valley fever virus in Sudan outbreak, 2007. *BMC infectious Diseases*, *10*, 1-8.

Sharma, A. (2009). *<textbook-of-veterinary-virologypdf-pr_c9194dd6c505a9bb35c49ffb47ab5176.pdf>*.

Shartova, N., Mironova, V., Zelikhina, S., Korennoy, F., & Grishchenko, M. (2022). Spatial patterns of West Nile virus distribution in the Volgograd region of Russia, a territory with long-existing foci. *PLoS Negl Trop Dis*, *16*(1), e0010145. https://doi.org/10.1371/journal.pntd.0010145

Shoemaker, T. R., Nyakarahuka, L., Balinandi, S., Ojwang, J., Tumusiime, A., Mulei, S., Kyondo, J., Lubwama, B., Sekamatte, M., Namutebi, A., Tusiime, P., Monje, F., Mayanja, M., Ssendagire, S., Dahlke, M., Kyazze, S., Wetaka, M., Makumbi, I., Borchert, J.,…Lutwama, J. (2019). First Laboratory-Confirmed Outbreak of Human and Animal Rift Valley Fever Virus in Uganda in 48 Years. *Am J Trop Med Hyg*, *100*(3), 659-671. https://doi.org/10.4269/ajtmh.18-0732

Shumbahri, M., & Idris, M. (2021). Dryland forest resource and management practices in Afar Regional State. *A review*, 101.

Sindato, C., Mboera, E., & Karimuribo, E. (2012). The epidemiology and socio-economic impact of Rift Valley fever epidemics in Tanzania: A review. *Onderstepoort Journal of Veterinary Research*, *79*(2), 1.

Sonneveld, B., van Wesenbeeck, C., Keyzer, M., Beyene, F., Georgis, K., Urbano, F., Meroni, M., Leo, O., Yimer, M., & Abdullatif, M. (2017). Identifying Hot Spots of Critical Forage Supply in Dryland Nomadic Pastoralist Areas: A Case Study for the Afar Region, Ethiopia. *Land*, *6*(4). https://doi.org/10.3390/land6040082

Sule, W. F., Oluwayelu, D. O., Hernández-Triana, L. M., Fooks, A. R., Venter, M., & Johnson, N. (2018). Epidemiology and ecology of West Nile virus in sub-Saharan Africa. *Parasites & vectors*, *11*, 1-10.

Suu-Ire, R. D., Obodai, E., Bonney, J. H. K., Bel-Nono, S. O., Ampofo, W., & Kelly, T. R. (2021). Viral Zoonoses of National Importance in Ghana: Advancements and Opportunities for Enhancing Capacities for Early Detection and Response. *J Trop Med*, *2021*, 8938530. https://doi.org/10.1155/2021/8938530

Tajudeen, Y. A., Oladipo, H. J., Oladunjoye, I. O., Yusuf, R. O., Sodiq, H., Omotosho, A. O., Adesuyi, D. S., Yusuff, S. I., & El-Sherbini, M. S. (2022). Emerging arboviruses of public health concern in Africa: priorities for future research and control strategies. *Challenges*, *13*(2), 60.

Tamiru, Y., Abdeta, D., & Amante, M. (2022). Knowledge, Attitude, and Practice Toward Pet Contact Associated Zoonosis in Western Ethiopia. *Vet Med (Auckl)*, *13*, 47-58. https://doi.org/10.2147/VMRR.S346806

Taylor, D., Hagenlocher, M., Jones, A. E., Kienberger, S., Leedale, J., & Morse, A. P. (2016). Environmental change and Rift Valley fever in eastern Africa: projecting beyond HEALTHY FUTURES. *Geospat Health*, *11*(1 Suppl), 387. https://doi.org/10.4081/gh.2016.387

Tennant, W. S. D., Cardinale, E., Cetre-Sossah, C., Moutroifi, Y., Le Godais, G., Colombi, D., Spencer, S. E. F., Tildesley, M. J., Keeling, M. J., Charafouddine, O., Colizza, V., Edmunds, W. J., & Metras, R. (2021). Modelling the persistence and control of Rift Valley fever virus in a spatially heterogeneous landscape. *Nat Commun*, *12*(1), 5593. https://doi.org/10.1038/s41467-021-25833-8

Terasaki, K., Tercero, B. R., & Makino, S. (2016). Single-cycle replicable Rift Valley fever virus mutants as safe vaccine candidates. *Virus Res*, *216*, 55-65. https://doi.org/10.1016/j.virusres.2015.05.012

Tigoi, C., Lwande, O., Orindi, B., Irura, Z., Ongus, J., & Sang, R. (2015). Seroepidemiology of selected arboviruses in febrile patients visiting selected health facilities in the lake/river basin areas of Lake Baringo, Lake Naivasha, and Tana River, Kenya. *Vector Borne Zoonotic Dis*, *15*(2), 124-132. https://doi.org/10.1089/vbz.2014.1686

Tigoi, C., Sang, R., Chepkorir, E., Orindi, B., Arum, S. O., Mulwa, F., Mosomtai, G., Limbaso, S., Hassan, O. A., Irura, Z., Ahlm, C., & Evander, M. (2020). High risk for human exposure to Rift Valley fever virus in communities living along livestock movement routes: A cross-sectional survey in Kenya. *PLoS Negl Trop Dis*, *14*(2), e0007979. https://doi.org/10.1371/journal.pntd.0007979

Tinto, B., Quellec, J., Cetre-Sossah, C., Dicko, A., Salinas, S., & Simonin, Y. (2023). Rift Valley fever in West Africa: A zoonotic disease with multiple socio-economic consequences. *One Health*, *17*, 100583. https://doi.org/10.1016/j.onehlt.2023.100583

Tofu, D. A., Fana, C., Dilbato, T., Dirbaba, N. B., & Tesso, G. (2023). Pastoralists' and agro-pastoralists' livelihood resilience to climate change-induced risks in the Borana zone, south Ethiopia: Using resilience index measurement approach. *Pastoralism*, *13*(1), 1-14.

Tong, C., Javelle, E., Grard, G., Dia, A., Lacrosse, C., Fourié, T., Gravier, P., Watier-Grillot, S., Lancelot, R., & Letourneur, F. (2019). Tracking Rift Valley fever: from Mali to Europe and other countries, 2016. *Eurosurveillance*, *24*(8), 1800213.

Troupin, A., & Colpitts, T. M. (2016). Overview of West Nile virus transmission and epidemiology. *West Nile Virus: Methods and Protocols*, 15-18.

Troupin, A., & Colpitts, T. M. (2016). Overview of West Nile Virus Transmission and Epidemiology. *Methods Mol Biol*, *1435*, 15-18. https://doi.org/10.1007/978-1-4939-3670-0_2

Tshilenge, G. M., Mulumba, M. L. K., Misinzo, G., Noad, R., & Dundon, W. G. (2019). Rift Valley fever virus in small ruminants in the Democratic Republic of the Congo. *Onderstepoort J Vet Res*, *86*(1), e1-e5. https://doi.org/10.4102/ojvr.v86i1.1737

Tucker, C. J., Melocik, K. A., Anyamba, A., Linthicum, K. J., Fagbo, S. F., & Small, J. L. (2020). Reanalysis of the 2000 Rift Valley fever outbreak in Southwestern Arabia. *PLoS One*, *15*(12), e0233279.

Ulbert, S. (2011). West Nile virus: the complex biology of an emerging pathogen. *Intervirology*, *54*(4), 171-184. https://doi.org/10.1159/000328320

Ulloa, A., Ferguson, H. H., Mendez-Sanchez, J. D., Danis-Lozano, R., Casas-Martinez, M., Bond, J. G., Garcia-Zebadua, J. C., Orozco-Bonilla, A., Juarez-Ordaz, J. A., Farfan-Ale, J. A., Garcia-Rejon, J. E., Rosado-Paredes, E. P., Edwards, E., Komar, N., Hassan, H. K., Unnasch, T. R., & Rodriguez-Perez, M. A. (2009). West Nile virus activity in mosquitoes and domestic animals in Chiapas, Mexico. *Vector Borne Zoonotic Dis*, *9*(5), 555-560. https://doi.org/10.1089/vbz.2008.0087

Van den Bergh, C., Venter, E. H., Swanepoel, R., & Thompson, P. N. (2019). High seroconversion rate to Rift Valley fever virus in cattle and goats in far northern KwaZulu-Natal, South Africa, in the absence of reported outbreaks. *PLoS neglected tropical diseases*, *13*(5), e0007296.

Venkat, H., Yaglom, H. D., & Adams, L. (2019). Knowledge, attitudes, and practices relevant to zoonotic disease reporting and infection prevention practices among veterinarians - Arizona, 2015. *Prev Vet Med*, *169*, 104711. https://doi.org/10.1016/j.prevetmed.2019.104711

Venter, M., & Swanepoel, R. (2010). West Nile virus lineage 2 as a cause of zoonotic neurological disease in humans and horses in southern Africa. *Vector Borne Zoonotic Dis*, *10*(7), 659-664. https://doi.org/10.1089/vbz.2009.0230

Wegdan, H., Rayan, M., Mohamed, S., Mutwakil, S., SalmaOA, M. A., & Alsarraj, S. Seroprevalence of West Nile Virus in Equine and Chickens in Sudan.

Whiteman, A., Mejia, A., Hernandez, I., & Loaiza, J. R. (2018). Socioeconomic and demographic predictors of resident knowledge, attitude, and practice regarding arthropod-borne viruses in Panama. *BMC Public Health*, *18*(1), 1261. https://doi.org/10.1186/s12889-018-6172-4

Wilkerson, R. C., Linton, Y.-M., Fonseca, D. M., Schultz, T. R., Price, D. C., & Strickman, D. A. (2015). Making mosquito taxonomy useful: a stable classification of tribe Aedini that balances utility with current knowledge of evolutionary relationships. *PLoS One*, *10*(7), e0133602.

Wilson, W. C., Mitzel, D., Savini, G., Zientara, S., & Richt, J. A. (2020). Editorial: Emerging Arboviruses. *Front Vet Sci*, *7*, 593872. https://doi.org/10.3389/fvets.2020.593872

Wilson, W. C., Weingartl, H. M., Drolet, B. S., Dave, K., Harpster, M. H., Johnson, P. A., Faburay, B., Ruder, M. G., Richt, J. A., & McVey, D. S. (2013). Diagnostic approaches for Rift Valley fever. *Dev Biol (Basel)*, *135*, 73-78. https://doi.org/10.1159/000276828

Yismaw, M. (2021). Economic Justification of Debre Elias–Yewula Road Project, Ethiopia. *Journal of World Economic Research*, *10*(2), 28-36.

Yuseri, N. A. N. M., Rahaman, N. Y. A., & Omar, A. R. (2019). West Nile virus infection in human and animals: potential risks in Malaysia. *Sains Malaysiana*, *48*(12), 2727-2735.

Zerfu, B., Medhin, G., Mamo, G., Getahun, G., Tschopp, R., & Legesse, M. (2018). Community-based prevalence of typhoid fever, typhus, brucellosis and malaria among symptomatic individuals in Afar Region, Ethiopia. *PLoS Negl Trop Dis*, *12*(10), e0006749. https://doi.org/10.1371/journal.pntd.0006749

Zerga, B. (2015). Awash National Park: Its degradation status and protection measures. *Palgo Journal of Agriculture*, *2*(3), 57-66.

Zouaghi, K., Bouattour, A., Aounallah, H., Surtees, R., Krause, E., Michel, J., Mamlouk, A., Nitsche, A., & M'Ghirbi, Y. (2021). First Serological Evidence of Crimean-Congo Hemorrhagic Fever Virus and Rift Valley Fever Virus in Ruminants in Tunisia. *Pathogens*, *10*(6). https://doi.org/10.3390/pathogens10060769

8. ANNEXES

Annex 1: KAP, Questionnaire Survey Data Collection Tool

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Microbiology, Immunology and Veterinary Public Health

Data Collection based on a Questionnaire to assess the Knowledge, Attitude and Practices of Community about Rift valley and West Nile Fever at Afar Regional State, Northeast Ethiopia

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By: Jemberu Alemu Megenas

Part I: Socio-demographic Characterization of the Study Participants

1. Code No/ Occupation: _____
2. District: _____
3. Sex: 1. Male 2. Female
4. Age: _____
5. Place of birth 1. Urban 2. Rural
6. Educational Status? 1. Illiterate 2. Primary School 3. Secondary and Above
7. Marital Status? 1. Married 2. Single 4. Others
8. Duration of stay in this area_____
9. Do you rear livestock? 1)Yes 2) No

Sn	Number of Animals Kept				
Species	Cattle	Goat	Sheep	Camel	Equines
Number/herd size					

Part II. Knowledge towards rift valley fever and West Nile fever

1. Have you ever observed diseases outbreak in your herds/locality? 1) Yes 2) No
2. If "yes" question no.1 above, what signs and/or symptoms you observed during the event?

   1) Sudden death 2) sudden onset of extensive abortion 3) smelly and blood-stained diarrhea 4) excessive salivation and watery discharge on the eyes of mature animals 5) Blood-stained Nasal discharge 6) Still birth 7) others, please specify……
3. Have you ever seen the sign of mass abortion in your herd? 1)Yes 2) No
4. If "yes" question no. 3 above, in which species of animals? 1) Cattle 2) Sheep 3) Goat 4) Camels 5) Equines
5. If it happens, in which season does such event happened? 1) Dry season 2) Rainy season
6. If in "rainy seasons", was there history of heavy rainfall before the event? 1) Yes 2) No

7. Have you ever heard about Rift valley fever/West Nile fever (locally)? 1) Yes 2) No
8. If your answer is yes for number 7, from where did you hear? 1) from a friend 2) health professional 3) from radio (mass media) 4) I or/member of my family, animals have been sick 5) from another sick person 6) other _____
9. Do you know the signs and symptoms of Rift valley Fever in affected cattle, camels, goats, and sheep 1) Yes 2) No
10. If your answer is yes to number 9 could you please mention some of the main signs? 1) Sudden death 2) Abortion 3) Muscle and bone pain 4) Other_____
11. Did you know that Rift Valley Fever/West Nile fever viruses could be transmitted from animals to humans? 1) Yes 2) No 3) I do not know
12. If your answer is yes for the above (11), how? 1) through biting mosquitoes 2) with other biting fly 3) through ticks 4) through drinking raw milk 5) through contact with wild animals 6) contact with birds/poultry 7. Other_____
13. Are Rift valley and West Nile transmitted from one animal to another? 1) Yes 2) No
14. If yes for above (13) question through what? 1) through biting mosquitoes 2) with other biting fly 3) through ticks 4) contact with sick animals 5) Other_____
15. Do you know the impact of Rift Valley Fever on pregnant cattle/sheep/goat? 1) Yes 2) No
16. If your answer for above (15) is yes what are the main impacts? 1) Abortion 2) still birth 3) death of delivered young 4) organ injury
17. Do you know other hosts that could be affected with rift valley fever? 1) yes 2) No
18. If your answer is yes to the above (17) mention some of them? 1) wild animals 2) monkey/Gorilla 3) Human 4) others

Part 3: Participants' attitude about Rift Valley Fever and West Nile fever

1. Are Rift valley and West Nile fever disease a newly occurred disease in this area? 1) Yes 2) No 3) I do not know
2. Do you agree that Rift Valley fever is an animal's health issue in this area 1) yes 2) no 3) I am not sure
3. Habit of consumption of food of animal origin? 1) raw 2) cocked/boiled 3) fried
4. Have you ever encountered the following signs and symptoms? 1)fever 2) weakness 3) stomach pain 4) diarrhea 5) joint pains 6) headache 7)blurred vision or decreased vision 8) comma or seizure 9) back pain 10) maculopapular rash 11) pharyngitis
5. Do you think Rift Valley fever is a major public health issue in this area 1) Yes 2) No 3) I do not know

6. If yes in number 5, which symptom have you ever encountered? 1) sudden onset of flu-like symptoms 2) muscle pain 3) joint pains, and 4) headache with or without ocular disease 5) meningoencephalitis 6) hemorrhagic fever 7) all
7. Do you think Rift Valley and West Nile fever affects all age groups? 1) yes 2) no 3) I am not sure
8. Do you agree that Rift valley and West Nile fever viruses are a fatal public health problem? 1) yes 2) no 3) I am not sure
9. Is Rift valley fever a fatal animal disease? 1) yes 2) no 3) I am not sure
10. Is it simple to treat Rift Valley? 1) yes 2) no 3) I am not sure

Part 4: Participants Practice on Rift valley fever and West Nile fever

1. Have you ever participated in the following activities 1) herding 2) taking care of sick animals 3) assisting aborting/delivery animals 4) animal health workers 5) Abattoir worker/postmortem inspector 6) milker/meat cooker 7) drinking blood
2. Is there experienced of animals and human leaving in the same shelter/house? 1) yes 2) no
3. Do humans and animals share the same watering point? 1) Yes 2) No
4. If yes for question number 3, where? 1) stream 2) river 3) irrigation canal 4) pond /dam 5) lake 6) others, specify……...
5. Are there movements of animals from one site/locality to another within the country and out of the country? 1)Yes 2) No
6. Is there common grazing land in your surroundings? 1)Yes 2) No
7. Is there a history of heavy rainfalls for 1-2 weeks which led to flooding in your surrounding? 1) Yes 2)No
8. If yes, have you observed mosquitoes biting you or your animals ever? 1) yes 2) No
9. Have you ever observed 1-2weeks laying water associated with flooding from heavy rainfall in your area? 1) Yes 2) No
10. Do you have any travel history to other countries? 1) yes 2) no
11. If yes, where _____when _____
12. Do you have a history of residence in other countries? 1) yes 2) no
13. If yes, where_____ How long_____
14. Have you or your family encountered symptoms like transient fever and extensive bleeding? 1) yes 2) No
15. Do you or members of the family feel unsecure of being attacked by Rift Valley and West Nile fevers? 1) Yes 2) No 3) I am not sure
16. Is Rift Valley and West Nile being fatal diseases of animals in this area 1) yes, it is fatal 2) No it is not fatal 3) I am not sure
17. Do you think it is possible to prevent Rift valley and West Nile? 1)Yes 2) No 3) I do not know
18. If your answer is yes for above question (17) what the methods are

1) Prevent mosquito, fly, and tick biting
2) Avoiding contact with Monkey/Gorilla
3) Other (specify)_____

19. What are the preventing methods from mosquitoes biting? 1) Bed nets 2) Chemical spraying 3) Others
20. If your animals, you, or/and member of your families think of being infected by rift valley fever, what do you do?
    1) Go to health center/veterinary clinic
    2) Self-treatment at home
    3) Go to traditional healer
    4) Go to religious place
    5. Other_____

Data collectors full name _____

Signature_____

Date_____

Annex 2: Focus group discussion checklist.

Addis Ababa University

College of Veterinary Medicine and Agriculture

Study on Community Awareness about the Veterinary Public Health Significance of Rift Valley Fever Virus and West Nile Fever virus in Afar Regional States, Northeastern Ethiopia

Data Collection Based on a Focal Group Discussion

Moderator (researcher) will guide the discussants on the following topics

Dear participants

Please feel free to discuss and give what you know on the following points.

In this group discussion, the researcher/coordinator will organize the study participants from different villages (8-10 persons of males and females, young and adults) to a common place for group discussions.

Data collection tools will consist of mainly focus group interviews. Interviews will be conducted by teams of four interviewers comprising of the team leader (interviewer), a community mobiliser, a translator, and a recorder.

They discussed on the issues listed below.

Participants' knowledge

1. Can you mention diseases that are transmitted to animals through mosquitoes' bite in this area?
2. Do you know about rift valley fever or West Nile virus and its vector? 1) yes 2) no??
3. Mention the common clinical signs/symptoms of the diseases?
4. Do all mosquitoes (vectors they mentioned) you mentioned transmit these diseases?
5. When do the mosquitoes (vectors they mentioned) bite animals? (Night, day, both)
6. If so, do you think that some mosquitoes (vectors they mentioned) bite during night while other during the day, can you identify/mention some of them which bite :a) during night , b) day c) both
7. Where do the mosquitoes (vectors they mentioned) bite? (inside home, outside home, under the shade, at any place)
8. Which mosquitoes (vectors they mentioned) transmit which diseases? Are the same mosquitos/vectors transmitting all these diseases? (accompanied by pictures/poster)
9. Breeding sites/places or seasons for the mosquitoes on……)
10. Do you know a disease that transmits through mosquitoes and affects both animals and persons? 1) yes or 2) no

(here discussion may be directed specifically to what they mentioned, with similar approach)

11. Mention the signs and symptoms of these diseases? (Make table)

Name of the disease (locally)	Name of the disease (common English name if possible)	Causative agent	Sign and symptom	Affected group (male, female, young, adult, humans, animals…?	Transmitted by (mosquitoes, flies ticks, …..)	Local remedy

12. Do you think that these diseases (mentioned above) transmitted from animals to humans? (make table)

13. Do you know a/an animal/person/ who was sick/died of these diseases (they mentioned) in this area?
    - Where this animal/person was living/grazing/watering?
        - In the city/urban
        - In the farming area/land near to the forest/rural
    - Where this animal/person was working/stay/grazing?
        - In the city/urban
        - In the farming area/land near to the forest/rural
    - (travel history of that animal/person e.g to Djibouti, Somalia)
    - Have you been/or your relatives or your animal travelled across the boarders or a chance of mixing with other animals came from abroad or purchased from another country?

15) Have you ever heard a disease known as Rift valley fever?
16) Does this disease affect cow, goat and other animals?
17) Clinical symptoms/signs of Rift Valley fever?
18) Does it transmit from animal to animal?
19) Mention mode of transmission?
20. Is there a prevention method (option) for these disease (They mentioned)?
    a. Please mention the prevention methods? If there is a vaccination system, have you/your animals been vaccinated?

Name of the disease (locally)	Name of the disease (common English name if possible)	Prevention methods locally or in the health center (yes/no)	
		Traditional (Yes/no)	Health center (yes/no)

21. Do you know that these diseases (they mentioned) do also transmit from wild animals to domestic? Or vise versa?
    - How does it transmit?
    - If you mention mosquito bite (the vector they may mention), which type of mosquito (the vector they mentioned), biting time and where?

Name of the disease (locally)	Name of the disease (common English name if possible)	Transmit from wild to to human/animals? (yes/no)	Vector/route (mosquitoes, flies, ticks…)	Biting time(day/night)	biting place (home/shelter or anywhere)

Attitude of the participants

1. Do you think that these diseases (the disease they mentioned) are a common public health and veterinary health problem of this area?
2. Do you think that these diseases (they mentioned) are killer in animals and humans?
3. Do you think treatment is easy for these diseases (they mentioned)?

Name of the disease (locally)	Name of the disease (common English name if possible)	Are they common public health problem(yes/no)	Are they common veterinary health problems	Are these diseases are killer (yes /no)	Is the treatment is simple/easy for these diseases (yes/no)

Participants practice about the diseases in the area

1. Is it easy to prevent this disease in animals? (they mentioned)?
2. Can you mention prevention methods for arthropod-borne viruses? (categorized) (they mentioned))
3. Is there a regular chemical spraying practice of your animals/home/shelter?
4. Is there a regular vaccination for arthropod borne viruses in the area?((they mentioned))
5. Do you use traditional healing practices for these diseases? (they mentioned))
6. Do you you track your animals to neighboring countries and mix them with other herds there?
7. Do you think you, member of your families, or livestock have been affected? and what did you done to treat the case(They mentioned))

Name of the disease (locally)	Name of the disease (common English name if possible)	Is it easy to prevent these diseases (Yes/no)

Additional information

1. Have you ever heard of other diseases that are transmitted to humans through mosquitoes' bite? 1) yes 2) No
2. Please mention some of the diseases transmitted through mosquitoes' bite.
3. Have these diseases recently occurred here?
4. Do you expect the presence of yellow fever, rift valley fever, dengue fever, Zika virus, dengue fever, etc

Annex 3: Livestock Information Registration Sheet

District: _____

S/N	Code	Woreda	Kebele	Species	Sex	Age	Bcs	Body Temperature	Tick Infestation	Illness Level	History of Abortion	Remark
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												

Annex 4:Consent Form

Date ----------

Name of study participant ---------- Age---------- Sex----------

Researcher Name---------- Site ----------

I have been informed about a study that plans to investigate the **Epidemiology of Rift Valley Fever Virus and West Nile Fever Virus in Livestock Population and their Public Health Importance in Afar Region, North Eastern Ethiopia**, which will help in understanding to determine the seroprevalence, molecular detection/virus isolations of RVF and WNV and to assess the current circulation of the diseases in livestock and human population of Afar Region, North Eastern Ethiopia. For this study I have been requested 5 ml of venous blood during the study period.

The investigator has briefed me that there are no major risks associated with the sampling procedure except very minimum bleeding and to avoid the possible risks, blood collection will be done by experienced health professionals according to the established aseptic procedure in clinical care. I have been informed that there is no direct benefit provided to me to participate in the study.

The investigator also informed me that all the laboratory results would be kept confidential. Moreover, I have also been well informed of my right to withdraw from participating in this project and that my actions will have no impact on the overall management of my conditions.

I have been given enough time to think over before I signed this informed consent. It is therefore, with full understanding of the situation that I gave my informed consent and cooperate at my will in the course of the conduct of the study.

Name (participant) ----------Signature ----------Date ----------

Name (investigator) ----------Signature ----------Date ---------

Name (Witness) ----------Signature ----------Date ----------

Annex 5: RVF-Testing Procedure

Allow all the reagents to come to room temperature (21°C ± 5°C) before use. Homogenize all reagents by inversion or Vortexing.

1. In the ELISA microplate Add:
   - 50 µl of Dilution Buffer 19 to each well.
   - 50 µl of the Positive Control to wells A1 and B1.
   - 50 µl of the Negative Control to wells C1 and D1.
   - 50 µl of each sample to be tested to the remaining wells.
2. Cover the plate and Incubate 1 hour ± 6 min at 37°C (± 2°C).
3. Empty the wells. Wash each well 3 times with approximately 300 µl of the Wash Solution. Avoid drying of the wells between washings.
4. Prepare the Anti-RVF-NP Conjugate 1X by diluting the Anti-RVF-NP-Po Conjugate 10X to 1/10 in Dilution Buffer 19.
5. Add 100 µl of the Conjugate 1X to each well.
6. Incubate 30 min ± 3 min at 21°C (± 5°C).
7. Empty the wells. Wash each well 3 times with approximately 300 µl of the Wash Solution. Avoid drying of the wells between washings.
8. Add 100 µl of the Substrate Solution to each well.
9. Incubate 15 min ± 2 min at 21°C (± 5°C) in the dark.
10. Add 100 µl of the Stop Solution to each well in the same order in step 8 to stop the reaction.
11. Read and record the O.D. at 450 nm.

Annex 6: West Nile Virus testing Procedure

Allow all the reagents to come to room temperature (21°C ± 5°C) before use. Homogenize all reagents by inversion or Vortexing.

1. In the ELISA microplate, Add:
   - 50 µl of Dilution Buffer 2 to each microwell.
   - 50 µl of the Positive Control to wells A1 and B1.
   - 50 µl of the Negative Control to wells C1 and D1.
   - 50 µl of each sample to be tested in the remaining wells.
2. Cover the plate and incubate 90 min ± 6 min at 21°C (± 5°C).
3. Empty the wells. Wash each well 3 times with approximately 300 µl of the Wash Solution. Avoid drying of the wells between washings.
4. Prepare the Conjugate 1X by diluting the Concentrated Conjugate 10X to 1/10 in Dilution Buffer 2.
5. Add 100 µl of the Conjugate 1X to each well.
6. Cover the plate and incubate 30 min ± 3 min at 21°C (± 5°C).
7. Empty the wells. Wash each well 3 times with approximately 300 µl of the Wash Solution. Avoid drying of the wells between washings.
8. Add 100 µl of the Substrate Solution to each well.
9. Cover the plate and incubate 15 min ± 2 min at 21°C (± 5°C) in the dark.
10. Add 100 µl of the Stop Solution to each well in the same order in step 8 to to stop the reaction.
11. Read and record the O.D. at 450 nm.

Annex 7: Published articles

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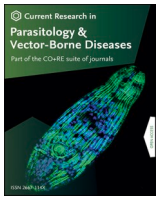

Seroprevalence of Rift Valley fever and associated risk factors in livestock of Afar Region, northeastern Ethiopia

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ABSTRACT

Rift Valley fever (RVF) is one of the emerging arthropod-borne zoonotic viral diseases with serious public and economic significance in the livestock and human populations of East Africa. Its epidemiology is inadequately recognized in Ethiopia. A cross-sectional study was conducted to investigate the seroprevalence and potential risk factors of RVF in domestic livestock of Amibara and Haruka districts of the Afar Region, northeastern Ethiopia. A total of 736 (224 cattle, 121 goats, 144 sheep, 155 camels and 92 donkeys) blood samples were collected, and serum extracted and tested using competitive ELISA. A questionnaire survey was used to assess potential risk factors of RVF infection. The overall seroprevalence was 22.0% (162/736; 95% CI: 19.41–24.79%). The seroprevalence was significantly higher in goats (42.2%, 95% CI: 39.61–44.99%) compared to that of cattle (14.3%, 95% CI: 11.74–17.09%), sheep (21.5%, 95% CI: 18.91–24.29%), or camels (30.97%, 95% CI: 28.38–33.76%) ($P < 0.001$). The study showed that seropositivity for IgG antibody to RVFV infection was associated with locality and species of animal. Goats were two times more likely to be seropositive for RVFV infection than cattle (OR: 2.3, 95% CI: 1.462–3.574, $P = 0.001$). Livestock in the Kealatburi area were five times more likely to be seropositive for RVFV infection than those in the Halidegei area (OR: 5.074, 95% CI: 3.066–8.396, $P = 0.001$). This study revealed that RVF is an important animal health problem in the Afar Region. Therefore, monitoring of RVF in animals, humans, and vectors along with community sensitization of high-risk populations could benefit mitigating the risk posed by the disease. Quarantine measures should be implemented to reduce the risk of RVFV introduction and dissemination among susceptible animals and ultimately transmission to humans.

1. Introduction

Ethiopia has the largest livestock population in Africa and several livestock diseases are endemic there. Given the large livestock population and distribution in the country and poor supply of veterinary services, various infectious diseases cause death and debilitation to a significant number of animals (Gutu et al., 2021; Jaleta et al., 2022). Arthropod-borne viruses (arboviruses) constitute important emerging and re-emerging infectious disease agents which pose substantial threats to animals and human health globally (Suu-ire et al., 2021). Rift Valley fever (RVF) is an arthropod-borne disease, mainly affecting a wide variety of livestock including cattle, small ruminants, and camels. The disease significantly affects livelihoods and national economy of the country (Hassan et al., 2020; Gibson et al., 2023).

Rift Valley Fever is becoming one of the important health issues with significant potential to emerge as a global concern. Within Africa and the Middle East, there are conditions favoring vector populations that are capable of transmitting the disease (Sindato et al., 2022). Competent vectors are known to exist even beyond the current range of RVF endemic areas and there is a recognized risk of global spread (Himeidan, 2016). RVF outbreaks in humans are preceded by epizootics in livestock (Kim et al., 2021). However, most of the major outbreaks have first been

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Article

Seroprevalence of West Nile Fever and Associated Risk Factors in Livestock of Afar Region, Northeast Ethiopia

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Simple Summary: Our study assessed the seroprevalence of West Nile virus (WNV) infection in domestic animals in the Amibara and Haruka districts of Ethiopia's Afar pastoral region, testing 736 serum samples from camels, cattle, donkeys, goats, and sheep. The overall seroprevalence of WNV IgG antibodies was 50.7%, with donkeys showing the highest prevalence (76.1%), followed by camels (69%), cattle (52%), goats (34.7%), and sheep (25.7%). These findings revealed a significantly higher prevalence compared with earlier studies in Ethiopia and other pastoral regions worldwide. Geographical differences, favorable vector breeding conditions, and temperature were identified as key factors influencing transmission dynamics. Risk factors such as species, sex, age, and location were examined, with species emerging as the most significant predictor of seropositivity. Female animals showed slightly higher seroprevalence, and older animals exhibited lower rates. The study underscores the importance of domestic livestock as sentinels for WNV surveillance, emphasizing the implications for both animal and human health in the region. These findings provide critical insights into the transmission of WNV, species-specific variations, and the environmental factors driving its prevalence in northeast Ethiopia.

Abstract: Sub-Saharan Africa has long been prone to widespread mosquito-borne diseases affecting both humans and animals. However, the presence and impact of West Nile virus (WNV) among livestock in Ethiopia have not been thoroughly investigated. The objective of this study was to investigate the seroprevalence of West Nile virus in livestock in the Afar region using serological methods. A total of 736 serum samples were collected from 224 cattle, 155 camels, 144 sheep, 121 goats, and 92 donkeys in the Amibara and Haruka districts of the Afar region selected using haphazard sampling. Among 736 tested livestock serum samples, 50.7% (373/736) showed anti-WNV IgG antibodies evaluated using the ID screen® WNV competition multispecies ELISA kits (95% CI: 47–54.4%; $p < 0.01$). The seroprevalence was higher ($p < 0.01$) in donkeys (76.1%), followed by camels (69.1%), cattle (52.2%), goats (34.7%), and sheep (25.7%). The study showed a statistically significant difference of WNV seropositivity between species of animals AOR (1.5), 95% CI (1.038–2.212) ($p < 0.01$). Compared with sheep, donkeys had a seven-fold higher chance of being seropositive for WNV infection (OR: 6.447, 95% CI = 3.888–10.688) ($p < 0.01$). This study emphasizes how common WNV infection is in Ethiopia's pastoral Afar region. It is imperative to consider consistent surveillance of WNV infection and prompt management of identified WNV disease in clinical practice. A clear need exists to build additional research capacity regarding WNV infections among both humans and animals.

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Research Article

Seroprevalence and Co-Circulation of Rift Valley Fever Virus and West Nile Fever Virus in Livestock Population of Afar Region, Northeast Ethiopia

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Abstract: The distribution, epidemiology, and socioeconomic impact of Rift Valley fever (RVF) and West Nile (WN) viruses are poorly known in areas of sub-Saharan countries like Ethiopian pastoral region. The human and livestock density in the area has increased greatly in recent years, but little work has been done on arboviral diseases and their potential impact on human and livestock health. The aim of this study was to detect the circulation of zoonotic arboviruses such as Rift Valley fever virus and West Nile viruses in the livestock population and to estimate seroprevalence in Afar pastoral area northeast Ethiopia. Cross-sectional serological survey was carried out in 736 serum samples from which cattle (224), camel (155), goats (121), sheep (144), and donkeys (92) were tested for the presence of anti-RVFV and anti-WNV IgG using a competitive enzyme-linked immunosorbent assay (c-ELISA) in two purposively selected districts of the Afar region. The present study revealed a 9.1% (95% CI = 8.86–9.29) sero-prevalence of co-circulation of RVF and WNV. High 32/155 (20.7%) seroprevalence of co-circulation was seen in camels, followed by goat 14/121 (11.6%), cattle 16/224 (7.14%), and sheep 5/144 (3.5%), respectively, and higher 41/421 (9.7%) seroprevalence of co-circulation was observed in *Amibara* district than *Haruka* district. Camels were seven times (OR: 7.016, 95% CI = 2.639–18.653) more likely to be seropositive for the co-circulation than sheep ($p \leq 0.001$). Livestock herds found in *Amibara* district were 1.2 times (OR: 1.165, 95% CI = 0.680–1.996) more likely to be seropositive for RVFV infection than those in *Haruka* areas. Given the co-occurrence of RVFV and WNV circulations, along with often suboptimal human and animal health surveillance in many similar areas' attention should be given. Investigation of the potential socioeconomic and health impacts of zoonotic arbovirus infections in such areas is crucial. Since both RVFV and WNFV are transmitted through a mosquito vector, avoiding mosquito bites is the primary method of prevention.

1. Introduction

Members of the families Flaviviridae, Togaviridae, Phenuviridae, Peribunyaviridae, Reoviridae, Asfarviridae, Rhabdoviridae, Orthomyxoviridae, and Poxviridae are among the diverse group of vector-borne pathogens known as arboviruses [1]. The most significant viruses that infect vertebrates are those belonging to the Bunyaviridae family which causes Rift Valley fever and Flaviviridae families which causes West Nile fever. These diseases are emerging or re-emerging in the 21st century and are extremely important for veterinary and public health [2, 3].

Rift Valley fever is a vector-borne zoonotic disease caused by the Rift Valley fever virus (*Phlebovirus* genus),

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RESEARCH ARTICLE

Knowledge, attitudes, and practices regarding Rift Valley fever and West Nile fever among livestock owners and health professionals in selected districts of the Afar Region, Northeast Ethiopia

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ABSTRACT

Objectives: East Africa is a hotspot for newly emerging arboviral diseases. In nations with limited resources like Ethiopia, outbreaks of vector-borne diseases can overwhelm healthcare systems. This study aimed to evaluate the knowledge, attitudes, and practices of health professionals, and livestock owners in selected areas of the Afar region. A cross-sectional study was employed between June 2021 and April 2022, using questionnaires, key informant interviews, and focus group discussions as data collection tools.

Materials and Methods: A total of 150 (60 livestock owners, 40 animal health workers and para-veterinarians, and 50 public health professionals) participated in the study.

Results: Of the participants, 29.3% (44/150) perceived zoonotic diseases to be transmitted through the consumption of food of animal origin or mosquito bites. More than half (63.3%, 95/150) of the participants had any feeling of insecurity regarding infection by Rift Valley fever (RVF), West Nile fever, or other zoonotic diseases. Animal health workers had higher (27.3±10.9) mean scores of knowledge of RVF, West Nile fever, and another zoonotic disease than public health professionals and livestock farmers/owners at $p < 0.01$. A statistically significant difference in mean scores was also observed among the educational status groups: illiterate, primary education (grades 1–8), and secondary and above (grades 9+). The mean practice scores were higher in the Amibara district (16.9 ± 6.4) compared to the Haruka district, with a p-value of 0.000.

Conclusion: The present study found substantial knowledge gaps, a low level of risk concern, and high behavioral practices regarding zoonotic diseases. Community education and awareness programs need to be developed, and further investigations into the prevalence and risk factors for zoonosis in such settings should be conducted to identify intervention targets.

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Attitude; Ethiopia; knowledge; practice; Rift Valley fever; West Nile fever; Zoonoses

Introduction

Maintaining animal health is becoming more and more crucial for the growth and welfare of human populations worldwide in light of demographic changes, increased international trade, and the effects of climate change [1]. In the Horn of Africa, vector-borne illnesses still impede the growth of public health, food security, and agricultural production. Around 1,500 arboviruses (arthropod-borne viruses) are known to be spread by 100 vector mosquito species worldwide. Most arboviruses are primarily spread by these mosquitoes [2]. Public health and economic

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REVIEW ARTICLE

SEROPREVALENCE AND ASSOCIATED RISK FACTORS OF RIFT VALLEY FEVER AND CRIMEAN CONGO HEMORRHAGIC FEVER VIRUSES IN LIVESTOCK AND THEIR ZOONOTIC POTENTIALS: A SYSTEMATIC REVIEW

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ABSTRACT

A systematic review was carried out to identify scientific articles documenting on seroprevalence of Rift Valley fever and Crimean Congo hemorrhagic fever infections of livestock and humans known to date to cause disease with associated risk in articles published from 2011 to 2021. Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (PRISMA) 2020 expanded checklist were used to summarize the article selection process. Search strategy was developed to retrieve eligible studies from PubMed (Medline)/OVOID, Animal Health and Production Compendium (CABI) and SciQuest (NZVA). The preliminary search retrieved 2,213 articles on the specified areas: 1,515 articles on Rift Valley Fever Virus and 698 articles on Crimean-Congo Haemorrhagic Fever Virus. Finally, a total of 106 (64 on RVFV and 42 on CCHFV) openly available articles were retained for the review process from 30 and 23 different countries, respectively. The articles were clustered into three clusters: articles on livestock sera, articles on human sera and articles from both human and different livestock (cattle, sheep, goat and camel) serum. The systematic review showed the pooled seroprevalence of RVFV (13.16%) and CCHFV (9.6%). The descriptive statistical analysis revealed statistical difference in the seroprevalence of Rift Valley fever in sheep, goat and camel ($\chi 2$=771.857840, 787.903297, 358.448980; p-value=0.002971*, 0.008652*, 0.000000**), respectively in different countries. In conclusion, this systematic review reflects circulation of RVF and CCKF in the world with complex ecology of vectors and the establishment of sustained transmission and the emergence of human and/or animal disease influenced by multiple factors.

Keywords: CCHF**,** RVF, risk factors, seroprevalence, zoonose

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