SEROPREVALENCE, RISK FACTORS IN CATTLE AND MOLECULAR INVESTIGATION IN TICKS OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS (CCHFV) IN CENTRAL MALAWI

BY

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A dissertation submitted to the University of Zambia in partial fulfillment of the requirements for the award of a degree of Master of Science in One Health Analytical Epidemiology

THE UNIVERSITY OF ZAMBIA

LUSAKA

DECLARATION

I, **Marvin Collen Phonera**, do hereby declare to the best of my knowledge, that the contents of this dissertation are my original work and have not been previously presented for the award of a degree in this University or any other institution.

Signature:…….Date……

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CERTIFICATE OF APPROVAL

This MSc dissertation of **MARVIN COLLEN PHONERA** has been approved as fulfilling the requirements for the award of the degree of Masters of Science in One Health Analytical Epidemiology by the University of Zambia.

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ABSTRACT

Crimean- Congo Hemorrhagic Fever virus (CCHFV) is zoonotic, causing subclinical infections in animals but fatal infections in humans. The virus is endemic in Africa, Asia, and Eastern Europe and its distribution corresponds to that of its principal vector, *Hyalomma* ticks. In Malawi, no case of CCHF has been reported and there is no evidence of CCHFV circulation in the country despite the presence of *Hyalomma* ticks. This study aimed to investigate the epidemiology of CCHFV in traditional cattle herds and ticks in central Malawi. A cross-sectional study was conducted in April 2020 in seven districts of central Malawi. Sera were collected from 416 randomly selected cattle (from 117 herds) and screened for CCHFV specific antibodies using a double antigen sandwich ELISA test. Ticks were collected from cattle and screened for the presence of the CCHFV genome using nested RT-PCR. Data on associated risk factors for CCHFV exposure in cattle were collected from cattle farmers using a structured questionnaire. CCHFV nucleoprotein specific antibodies of 46.86% (195/416; 95% CI: 42.02-51.82) was determined. This seropositivity was significantly associated (significance level: *p*-value <0.25) with cattle age sex, presence of ticks, district, type of grazing land, cattle herd size, and source of cattle. In a binary logistic regression model (significance level: (*p*-value < 0.05), cattle from Lilongwe West (OR 2.7; 95% CI 1.191-6.037) and Ntchisi (OR 5.0; 95% CI: 1.383-18.111) were more likely to be infected with CCHFV compared to those from Mchinji. Cattle aged 25-48 months and >48 months, were two and three times more likely to be CCHFV infected (OR= 4.33; 95% CI: 2.196-8.533; OR= 4.229; 95% CI: 2.032-8.798), respectively compared to cattle of 1-12 months old. Female cattle were 2.5 times more likely to be CCHFV infected than males (OR= 2.478; 95% CI: 1.568-3.944). There was a strong association between cattle grazing in uplands and being CCHFV seropositive (OR=4.489; 95% CI: 1.799-11.2). Cattle infested with ticks were three times more likely to be CCHFV seropositive than those that had no ticks (OR= 3.206; 95% CI: 1.208- 8.509). *Amblyomma variegetum*, *Rhipicephalus appendiculatus*, *Rhipicephalus (Boophilus) decoloratus*, *Rhipicephalus (Boophilus) microplus*, and *Hyalomma truncatum* were identified from the collected ticks. CCHFV S-segment genome was detected in 64.44% (29/45) of the RNA pools extracted from the ticks. These results indicate higher exposure of cattle to CCHFV in the study area. Therefore, good cattle management practices and awareness of the existing risk rae required to reduce chances of contracting the deadly CCHFV among cattle-keeping communities.

Key words: Crimean Congo Hemorrhagic Fever Virus, Seroprevalence, Malawi, risk factors, traditional cattle, ticks

DEDICATION

This work is being dedicated to my lovely wife Veronica and children: Bryan, Clara, and Sheila for their perseverance and patience during my period of study.

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LIST OF ABBREVIATIONS

ADD	Agricultural Development Division
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AU-CTTBD	African Union – Center for Tick- and Tick-Borne Diseases
CVL	Central Veterinary Laboratory
CCHF	Crimean-Congo hemorrhagic fever
CCHFV	Crimean Congo hemorrhagic fever virus
CDC	Centers for Disease Control and Prevention
cDNA	Complement deoxyribonucleic acid
CE	Clathrin endocytosis
CFR	Case fatality rate
CFT	Complement fixation test
cRNA	Complement Ribonucleic Acid
DAHLD	Department of Animal Health and Livestock Development
DAHLD-AHC	DAHLD -Animal Health Committee
DHIS	District Health Information Systems
DRC	Democratic Republic of Congo
EDTA	Ethylene diamine tetra-acetic acid
EE	Early Endosome
ELISA	Enzyme Linked Immunosorbent Assay
ENA	Emergency Nutritional Assessment
EPA	Extension Planning Area
GC	Glycoprotein complex
HF	Hemorrhagic fevers
ID	Immunodiffusion
Ig	Immunoglobulin
IFA	Immunofluorescence Assay

JAICA	Japanese International Cooperation Agency
L	Large
M	Medium
MVB	Multivesicular bodies
mRNA	Messenger ribonucleic Acid
NATA	Nucleic acid amplification tests
NSVD	Nairo Sheep Disease Virus
NP	Nucleoprotein
NSO	National Statistical Office
OIE	World organization for animal health
OR	Odds Ratio
ORF	Open reading frame
RPHA	Reverse passive hemagglutination
S	Small
P	Probability (p-value)
pH	Power of hydrogen
RNA	Ribonucleic Acid
RNP	Ribonucleic protein
RT-PCR	Reverse Transcription -Polymerase Chain Reaction
UNZABREC	University of Zambia Biomedical Research and Ethical Committee
USA	United States of America
VHF	Viral hemorrhagic fever
WHO	World Health Organization
WHO- AFRO	World Health Organization- Africa Region
WHO- EMR	World Health Organization- East Mediterranean Region

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Crimean-Congo Hemorrhagic Fever (CCHF) is a fatal arboviral zoonotic disease of humans caused by the CCHF virus (CCHFV). The virus circulates naturally between ticks and several non-human mammals and avian species without causing clinical disease but, occasionally spills over to humans, causing fatal outbreaks (Verma and Khanna, 2011; Balinandi et al., 2018). About 88% of human infections are asymptomatic (WHO, 2018a). Animals get infected when ticks are getting a blood meal (Verma and Khanna, 2011). In humans, transmission is mainly through bites of infectious Hyalomma ticks, and direct contact with body fluids of infected viremic animals and humans (Verma and Khanna, 2011; Al-Abri et al, 2017; Harry Hoogstraal and Read, 2019). Clinical symptoms in humans range from simple fever to severe cases with multiple organ failure (Fajs et al., 2014) and death (case fatality rate; 10-50%) (Goswami et al, 2014; Mertens et al., 2015). Up to date, there is no approved curative chemotherapy nor vaccine for CCHF (Sorvillo et al., 2020; Bartolini et al., 2019; WHO, 2016; Bergeron et al., 2015; Escadafal et al., 2012).

Geographically, about 50 countries from across Africa, Asia, and Eastern Europe are considered CCHFV endemic (Nasirian, 2019). Concurrently, these countries are also infested with Hyalomma (H.) ticks, which are known vectors of the disease (Hoogstraal et al., 2019; Hawman and Feldmann, 2018). The World Health Organization (WHO, 2018) estimates the global annual CCHF burden to be between 10,000 to 15,000 infections, with 500 deaths and, almost three billion people at risk. Drivers of the spatial distribution of CCHF include long-distance live animal trade, habitat fragmentation, expansion of agricultural/cultivation lands, and increase in seasonal mean temperature (Vescio et al., 2012).

CCHF has proven to be a serious infectious occupational hazard among people working along the livestock production chain including farmers, animal handlers, abattoir workers, and veterinarians because of increased exposure to tick bites and viremic animal body fluids (Rehman, 2018; Al-Abri et al., 2017; Goswami et al., 2014). The human-to-human transmission increases the risk among health workers (Mehanda et al., 2018; Richards., 2015;

Verma and Khanna, 2011; Jauréguiberry et al., 2005). Furthermore, the risk of CCHF to travelers (tourists) is increasing among those visiting endemic developing countries, who upon return, subsequently introduce CCHF in free regions (Leblebicioglu et al., 2016).

The distribution of CCHF is expanding globally by emerging in what was considered regions and also increase in incidence (Spengler and Bente, 2017; Nasirian, 2019; Gale et al., 2012). In Africa, CCHFV infections have been reported across the continent in both animals and humans (Spengler et al, 2016; Maiga et al., 2017; Mangombi et al., 2020; Adam et al., 2013). Malawi's agricultural sector is transforming and the changes include tick control strategies in livestock, increased within and cross border animal movements, and expansion of cultivation and grazing areas into natural forests and marginal lands following human population increase (NSO, 2019; DAHLD, 2006; Mares, 1964; Munthali and Murayama, 2013). The concurrent existence of the competent vectors (Hyalomma ticks) and favorable risk factors (Pigott et al., 2017), suggests a high potential risk of CCHF emergence in Malawi. There is a lack of epidemiological information on CCHFV/CCHF in Malawi, both in human and animal populations. Hence, this study aimed at improving the current understanding of the epidemiology of CCHFV in cattle in the central part of the country through a cross-sectional study.

1.2 PROBLEM STATEMENT

CCHF is a global notifiable disease with a high impact on public health and welfare as a result of a high case fatality rate, lack of approved curative treatment, and vaccines (Sorvillo et al., 2020). In most developing countries, the disease is not monitored in human nor animal populations. As a result, severe outbreaks do occur which destabilize healthcare systems/services and loss of human lives (Munibullah et al., 2018). Malawi is a developing country that has over 62% of its human population being livestock farmers (Pica-Ciamarra et al., 2011). This means that a greater proportion of the Malawi population is at risk of CCHF if the CCHFV is present in the country taking into consideration the presence of competent vector tick in the country (Berggren, 1978). Countries such as the Democratic Republic of Congo (DRC), Mozambique, Tanzania, South Africa, Zimbabwe, and Namibia which are geographically close to Malawi, have once or more before reported CCHF (Richards et al.,

2015; Ince et al., 2014; Goswami., 2014; Muianga et al., 2017), thus increasing the probability of the virus circulating in Malawi. The unchecked animal movement across the borders of these countries further increases the likelihood of CCHFV spreading across the countries. Malawi has a record of poor disease surveillance, including poor laboratory diagnostic capabilities (Wu et al., 2018), hence there is a high chance of missing cases in the country. Preparedness of health systems for such a disease is essential to minimize the number of secondary infections and reduce the impact of outbreaks at large (Balinandi et al., 2018). Unfortunately, despite the high likelihood of CCHF emergence in the country, there was no information of CCHF in the country. Therefore, there was a need to understand the epidemiology of CCHF in Malawi.

1.3 JUSTIFICATION

There is limited information about the epidemiology of CCHF in sub-Saharan Africa, especially in those countries with no history of having reported the disease in humans. However, CCHF is one of the neglected diseases listed under the WHO priority diseases that require research (WHO, 2018b) and an immediately reportable disease in Africa (WHO-AFRO, 2010), including Malawi (Wu et al., 2018). There is a lack of epidemiological information about CCHFV in humans, animals, and ticks in Malawi. The knowledge gaps that exist include the current distribution of Hyalomma ticks, the existence of CCHFV in the country, biological risk factors both in animals and humans, ecological, socio-economic, and climatic potential risk factors. The seroprevalence of CCHFV in cattle has been reported to directly correlate with the CCHF seroprevalence in healthy human populations (Fajs., et al., 2014; Nasirian, 2019), making this study relevant to both animal and human health sectors. Thus, the information generated in this study is expected to fill some of the gaps mentioned above and enhance the understanding of the epidemiology of CCHF in the country. Therefore, this study provides information that is important in formulating evidence-based surveillance as well as prevention and control strategies of CCHF in Malawi.

1.4 RESEARCH QUESTIONS

The study was set to answer the following questions:

What was the seroprevalence of CCHFV in cattle in Malawi?

Was there CCHFV circulating in cattle and/ or ticks in Malawi?

What were the potential risk factors associated with the seroprevalence of CCHF in cattle in Malawi?

1.5 OBJECTIVES

1.5.1 MAIN OBJECTIVE

To investigate the epidemiology of CCHFV in traditional cattle and ticks in central Malawi.

1.5.2 SPECIFIC OBJECTIVES

The specific objectives of this study were:

To determine the seroprevalence of CCHFV specific antibodies in cattle in central Malawi.

To determine risk factors associated with CCHFV seropositivity in traditional cattle populations in central Malawi.

To conduct molecular screening for CCHFV in ticks in central Malawi.

CHAPTER TWO
2.0 LITERATURE REVIEW

2.1 HISTORICAL BACKGROUND OF CCHFV

As an ailment, CCHF has been traced back to 1100 AD in Tajikistan where a physician described a hemorrhagic condition which is now thought to be the first record of CCHF (Al-Abri et al., 2017). In the recent past (1944-1945), the current era of CCHF (then strange hemorrhagic fever) began with an outbreak in Crimea among military and agricultural communities. In Africa, the first incursion was in Belgium Congo, in 1956. However, the etiology of the Crimean and the Congo outbreaks was considered different, until 1967 when the causative agent was isolated from a patient in Uzbekistan and was noted to be antigenically similar to the viruses that caused outbreaks in both Crimea and Congo. Hence the names CCHFV and CCHF (Tipih and Burt, 2020; Al-Abri et al., 2017; Jauréguiberry et al., 2005). Butenko and colleagues in 1968, made the first successful culture and isolation of CCHFV in newly born (suckling) white mice. This method later became a prototype protocol for culturing and isolating CCHFV. Since 1944-1945, CCHF has increased its spatial distribution and public health significance, globally (Nasirian, 2020). In response, extensive studies have been done and policy frameworks are drawn. An example of such is the inclusion of CCHF in the WHO priority list of emerging pathogens needing urgent research and development attention (WHO, 2016). In 2013, 3426 cases, 541 deaths were reported for the period from 2003 to 2008 in PreMED only, with most cases reported in Turkey followed by Russia (Ince et al., 2014). In the endemic countries, the cases steadily continue to rise (WHO-EMR, 2021; Chauhan et al., 2020)

2.2 THE BIOLOGY OF CCHFV

CCHFV belongs to the genus Orthonairovirus, family Nairoviridae, in the order of Bunyavirales (Maes et al., 2019; Cajimat et al., 2017). The order Bunyavirales comprises negative-sense RNA viruses some of which cause infections in humans (symptoms ranging from flu-like to meningitis and hemorrhagic fevers), animals, birds, and plants (Schreur et al.,

2018). The family Nairoviridae is new in the virus taxonomy, and the species in this family formerly belonged to the Bunyaviridae family (Maes et al., 2019), and has three genera assigned to it and these are Orthonairovirus, Shaspivirus, and Striwavirus. Of interest in this family is Orthonairovirus that contains, among many species, two that are of notable public and animal health importance, CCHFV and Nairo Sheep Disease Virus (NSDV) (Maes et al., 2019). NSDV causes severe disease in sheep and goats, and mild flu-like illness in humans (Davies, 1997).

CCHFV has a tripartite, single-stranded, negative-sense (ambisense) ribonucleic acid (RNA) genome packed in a spherical capsid and lipid envelope (Bente et al., 2013) (Figure 2-1). The size of a complete CCHFV genome is 19.2kb (S=1.672 kb, M = 5.364 kb, and L = 12.150 kb) (Duh et al., 2008; Deyde, 2006). Nucleoproteins (N) bound with RNA dependent RNA polymerase (RdRp), encaspsidate the three viral genomic segments forming ribonucleoprotein (RNP), a molecule instrumental for viral replication (Schreur et al., 2018). Each of the segments encodes for a distinct viral protein component: the S segment for viral nucleocapsid proteins (NP), the M segment for polyproteins (precursor of GN and GC structural glycoproteins) and non-structural proteins (i.e. G38), and the L segment for viral RNA dependent RNA polymerase proteins (Lasecka and Baron, 2014; Bente et al., 2013; Deyde et al., 2006; Hewson et al., 2004). The composition of nucleotides in genomic sequences of these segments varies significantly; 20 %, 22 %, and 31% for S, L, and M segments, respectively; such that there is clustering in the topology of virus sequences from a geographical location (Deyde et al, 2006). The characterization of CCHFV based on S and L genomic segments has resulted in the classification of seven distinct genotypes, which correspond to particular global regions of origin, named as; Africa 1, 2, and 3; Asia 1 and 2; and Europe 1 and 2 (Nasirian, 2020; Shayan et al, 2015; Shahhosseini et al., 2017). Similarly, by comparison of gene evolution rates (Anagnostou and Papa, 2009), six clades/linages have been classified as clades I, II, III, IV, V, and VI (Nasirian, 2020; Shayan et al., 2019). Within these clades, over 22 strains of CCHFV have been characterized to date (Chen, 2013; Rickert-hartman and Folster, 2014; Hewson et al., 2004; Shahhosseini et al., 2017).

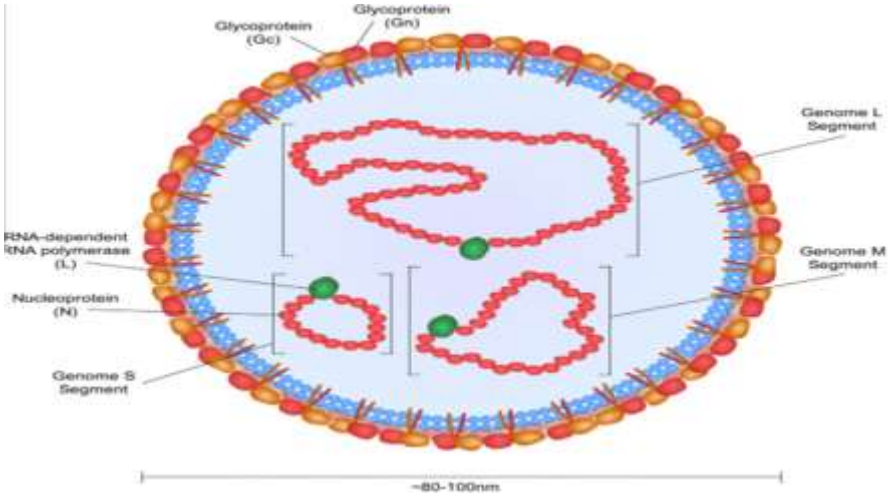

Figure 2-1: Schematic presentation of CCHFV virion and its components (Bente et al., 2013).

The CCHFV genome nucleotide sequence variation is thought to be a result of genetic re-assortment (Hewson et al., 2004) and recombination processes (Chinikar et al., 2016; Lukashev, 2005), as is the case in other segmented RNA viruses. Interestingly, the extent of re-assortment and recombination in CCHFV differ among the segments. Re-assortment is not uncommon in M segments, whereas recombination occurs in S segments. Phylogenetic classification of CCHFV based on L and S segments produces almost similar geographical clustering unlike with M segment. The S and L segment sequences of CCHFV are found to evolve with high fidelity unlike the M segment (Hewson, 2007; Deyde, 2006; Whitehouse, 2004).

The big question has been whether the genotypes of CCHFV have different virulence following significant differences in the number of clinical cases and case fatality rates (CFR) reported in different global regions (CFR: 22.0% (Africa), 33.5% (Asia), and 33.8% (Europe) (Nasirian, 2019). Coskun and Asik (2019) reported significant differences in levels of aspartate aminotransferase (AST), platelet, and activated partial thromboplastin time (APTT) in patients positive for one of the two genotype variants of CCHFV Europe V group, suggesting differences in virulence. Hawman and a colleague systematic literature review, could not resolve as to whether this virulence difference really existed or not (Hawman and Feldmann, 2018). A different hypothesis by Gonzalez, stated that host-virus interaction determines the virulence (Gonzalez, 1995). Garrison, et al, (2019), in their review, pointed out that genetic diversity's correlation to pathogenicity was poorly understood although some

genotypes of CCHFV were associated with high CFR in some endemic areas, a phenomenon that was presumably attributed to the interaction of viral strain, case management, and host factors.

2.2.1 VIRUS CELL ENTRY AND REPLICATION

Following sufficient exposure to CCHFV, the virus enters the extracellular compartment of the host and attaches to the host cell surface using its GN and GC surface proteins (Zivcec et al., 2016). However, the specific host cell receptors to which CCHFV attaches have not yet been identified (Garrison et al., 2013; Zivcec, et al., 2016). Host cell entry following attachment is much supported to be by Clathrin endocytosis mechanism (CEM), thus after demonstrations through Clathrin inhibitors experiments (Garrison et al., 2013). This endocytosis process is facilitated by Clathrin pit adaptor protein-2-complex in a low pH medium (Simon et al, 2009) and results in the formation of early endosome (EE) which contains virions. Early endosomes subsequently merge to form multivesicular bodies (MVBs) in the presence of Rab 5 proteins. The virion in the MVB is organized into a ribonucleic protein (RNP) complex which is formed by encapsidated nucleoprotein (NP), RNA dependent RNA polymerase (RdRp), and L protein that serves as a template for L protein to synthesize viral mRNA (Zivcec et al., 2016).

In the EE stages, the RNPs fuse with the host cell cytoplasmic membrane, a process that removes the virion coat, thus exposing the viral segments (Zivcec et al., 2016). With high affinity, NP binds to viral mRNA 5’ UTR initiating a downstream translation of reporter mRNA at the open reading frame (ORF) (Jeeva et al., 2017). Transcription of these segments by L proteins forms viral mRNA that snatches a 5' cap from the host cell in the presence of Mn2+. Then mRNA is translated into NP and L proteins by cytoplasmic ribosomes. Glycoprotein complex is translated by endoplasmic reticulum-associated ribosomes. The newly formed viral particles are used to replicate genomic RNA by the formation of an RNP that contains antigenic RNA (cRNA) (Zivcec et al., 2016). Glycoprotein complex is processed and cleaved into GN and GC in the endoplasmic reticulum and Golgi apparatus and further assembling of the nascent virion particles is done in the Golgi apparatus and released through Golgi derived vesicles (Zivcec et al., 2016).

Virion release is by exocytosis and egress occurs from the basolateral membranes in polarized epithelial cells (Garrison et al., 2013; Zivcec et al., 2016). Figure 2-2 shows the entry of the virus into a mammalian host cell using CEM, transcription, replication, and release of the new virus via exocytosis; and a completely assembled CCHFV.

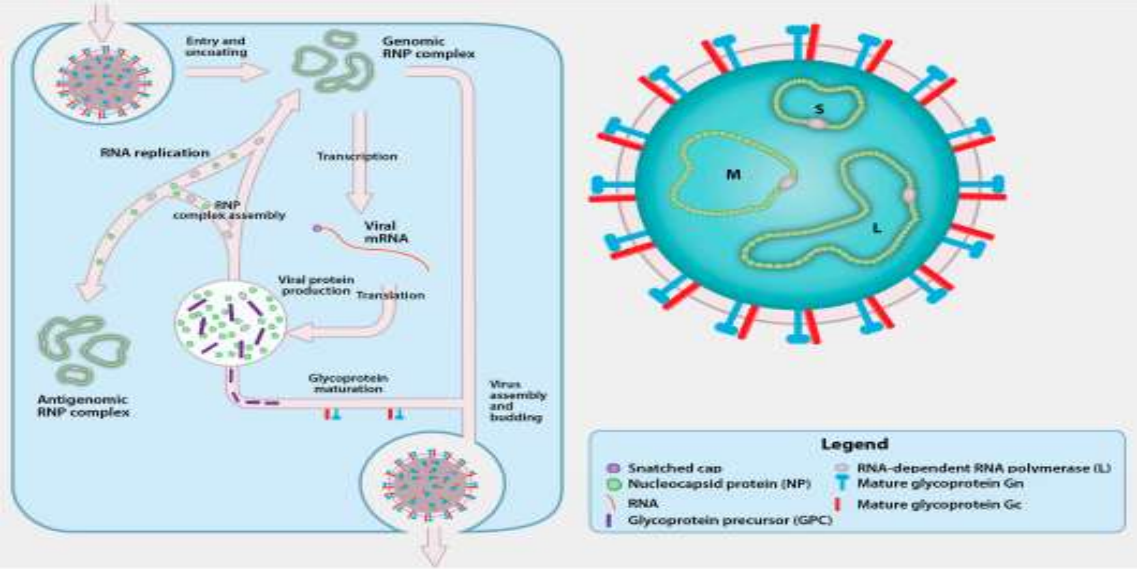

Figure 2-2: CCHFV host cell entry, multiplication, and release (Zivcec, 2016)

2.3 EPIDEMIOLOGY AND GEOGRAPHICAL DISTRIBUTION OF CCHFV

2.3.1 DISTRIBUTION OF CCHFV AND CCHF

CCHFV is the most known widely distributed arbovirus being found in 50 countries in the regions of Africa, Asia, and Eastern Europe(Nasirian, 2019). Interestingly, the global spatial distribution of CCHFV corresponds to the distribution pattern of its principal vector, Hyalomma ticks (Hoogstraal and Read, 2019; Hawman and Feldmann, 2018; Walker et al., 2014)and global spatial expansion of CCHFV is following the Hyalomma territorial expansion (Bartolini et al., 2019). The recent surveillance studies have reported the emergence of CCHFV in countries outside the previously known endemic regions: India and Spain are examples of such countries that have reported the incursion of CCHF in humans (Leblebicioglu, 2010; Mourya et al., 2012; Palomar et al., 2017). In the just ending decade, many African countries have reported serological evidence of CCHFV and virus detection in animals, humans, and/or ticks. These countries include Ghana, Morocco, Mozambique

(Akuffo et al., 2016; Muianga et al., 2017; Palomar et al., 2013). CCHFV has been known to be endemic in many African countries although CCHF human cases have been reported sporadically with exception of South Africa, Uganda, and Sudan where there has been a relatively consistent occurrence of the disease (Balinandi et al., 2018; Richards et al., 2015; Aradaib et al., 2011). Figure2-3 below shows the global distribution of CCHF. Note should be taken that Mozambique mentioned here is not included in this map.

The spread of CCHFV into new regions has been influenced by many factors, most notable ones being extreme climate change favoring tick proliferation, disasters (floods and droughts) that disturb tick-animal ecosystems, animal translocation through trade and human displacement, and agricultural expansion causing settlement fragmentation (Tipih and Burt, 2020; Hassanein et al; 1997; Hoogstraal, 1981).

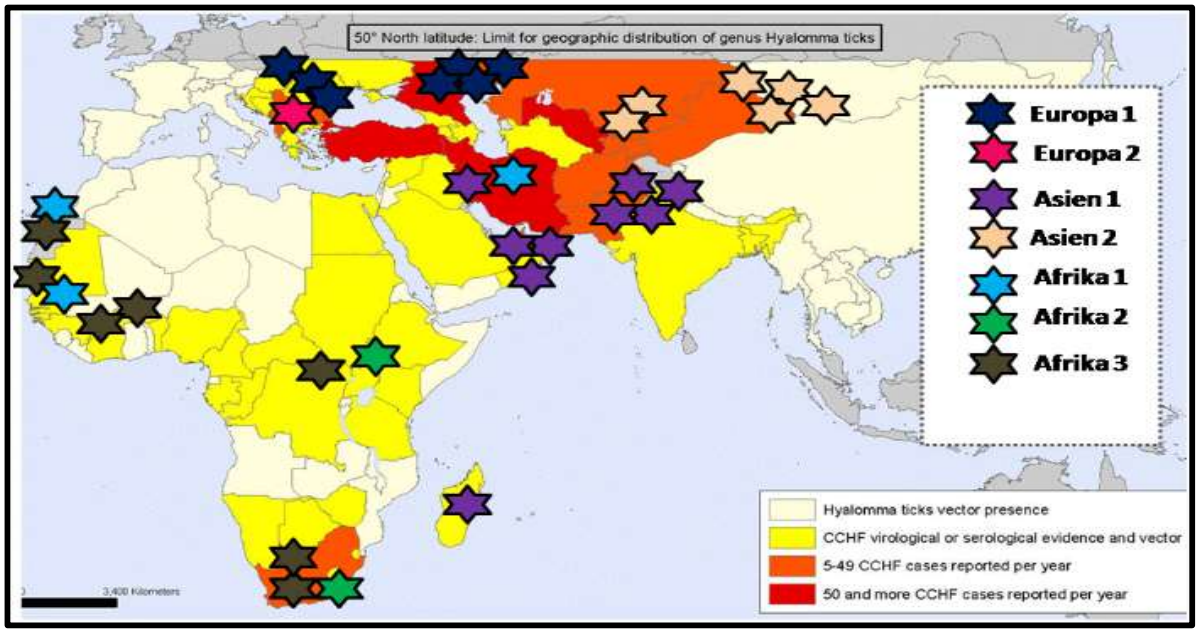

Figure 2-3: Global distribution of CCHFV clades and Hyalomma ticks (https://wehrmed.de/article/3466-haemorrhagic-fever-in-the-field.html. 19/07/2019)

2.3.2 DRIVERS AND RISK FACTORS OF CCHFV DISTRIBUTION

CCHFV has been expanding its geographical distribution since it was first reported and increasing in prevalence in the human and animal populations (Nasirian, 2019; Nasirian, 2020). Several factors have been implicated as drivers of the distribution of CCHFV in

endemic and also in new regions. Migratory birds were assumed to be carrying CCHFV infected ticks on them to distant regions as the case of CCHFV which was detected in Morocco and is assumed to have been disseminated by these birds to south-eastern parts of Europe (Palomar et al., 2013). Long-distance livestock trade has been shown to contribute towards the distribution of CCHFV as goats and sheep from Sudan to Saud Arabia for religious festivals were found seropositive for CCHFV on arrival (Hassanein et al., 1997). Migrating infected humans have also been reported to introduce CCHFV in free continents and countries as imported cases (Conger et al., 2015).

Environmental factors influence the distribution of CCHFV by altering the ecology of vector ticks. For instance, deforestation, settlement fragmentation and cultivation, changing pasture patterns, converting wildland into farmlands or pastures have been observed to be associated with the occurrence of CCHF cases (Vescio et al., 2012; Hoogstraal and Read, 2019). Seasonal variation also influences tick activity especially in the warm or hot season which facilitates transmission of the virus among animals and also humans (Sorvillo et al., 2020 Vescio et al., 2012; Mostafavi et al., 2013). The presence of ticks, small ground animals/birds, and livestock management (tick control) influences livestock and human exposure to tick bites (Hoogstraal and Read, 2019; Bente et al., 2013)

Among livestock, age has been consistently reported as a risk factor with old animals have been observed to be more likely to be infected or exposed to CCHFV when compared to young animals as an of repeated exposure to infected ticks bites (Adam, et al., 2013; Maiga et al., 2017; Balinandi et al., 2021). The sex of cattle has been reported as a risk factor to CCHFV positivity in some studies (Mangombi et al., 2020).

CCHFV infections among humans are largely an occupational-related hazard with people who work closely with animals or in fields infested with ticks being at high risk of exposure to CCHFV. These high-risk individuals include veterinarians, livestock farmers, abattoir workers, military personnel, shepherds, and hunters (Sorvillo et al., 2020; Al-Abri et al., 2017; Arteaga et al., 2020). Nosocomial transmissions render public health workers and hospital workers at risk of infection when caring for CCHF patients and handling potentially infectious

materials (Conger et al., 2015).

2.3.3 ROLE OF ANIMALS IN THE EPIDEMIOLOGY OF CCHF

Both wildlife and livestock are susceptible to CCHFV infection, and the larger animals show higher seropositivity (Burt et al., 1993), a phenomenon that has been attributed to cumulative exposure because of longer life spans compared to small animals. The seroprevalence is relatively higher in animals than humans, 24.6% against 4.7%, respectively; as reported in a global meta-analysis by Nasirian (2019). Although these animals do get asymptomatic infections, the virus do amplify the animals and later infect many other ticks (Spengler, 2019; Spengler et al., 2017; Fajs Humolli, et al., 2014; Adam et al., 2013; Telmadarraiy et al., 2010; Bakir et al., 2005). Migratory birds have been implicated in facilitating the spread of CCHFV by hosting immature ticks and flying with them to distant regions (Palomar et al., 2013).

Since the seroprevalence is high in animals than in humans, animals are commonly used as indicator hosts for CCHFV circulation in non-endemic regions (Mertens et al., 2015). Furthermore, it has been observed that seroprevalence in cattle positively correlated with seroprevalence in healthy human populations (Fajs., et al., 2014), making animal serological studies useful in gauging the risk of CCHF in human populations. The movement of animals may complicate the interpretation of serological test results data such as the location of detection may not always be the same as the location of exposure (Sorvillo et al., 2020). Figure 2-4 below shows the tick-vertebrate-tick cycle transmission pathways of CCHFV and how the virus jumps into the human population.

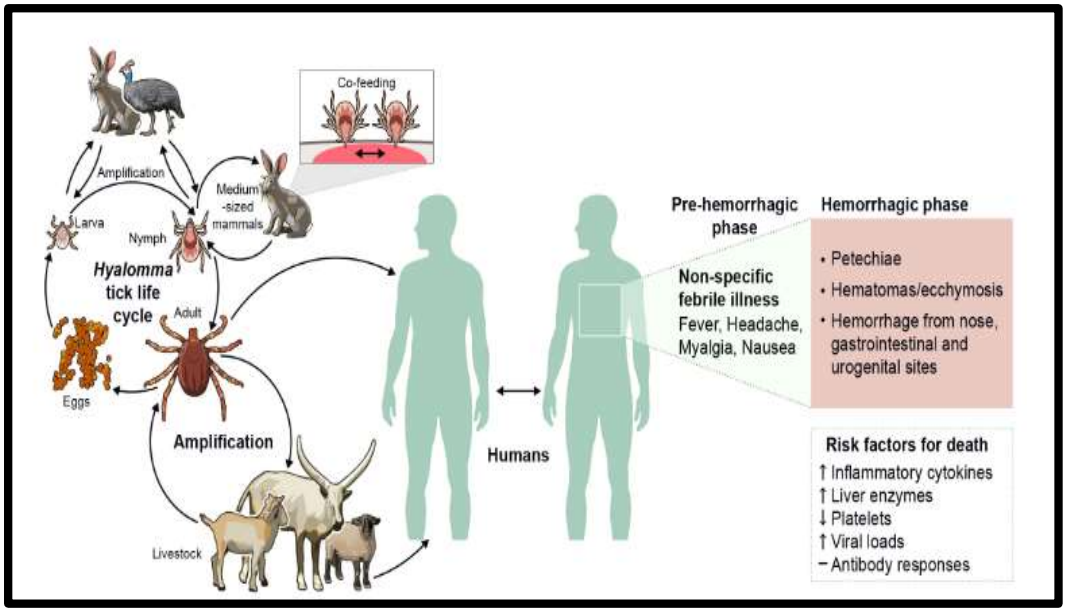

Figure 2-4: Tick- vertebrate -tick cycle for maintenance of CCHFV and spill over to humans (Hawman and Feldmann, 2018)

2.3.4 MAINTENANCE AND TRANSMISSION OF CCHV BY VECTOR TICKS

CCHFV has been detected in many species of both Argasidae and Ixodidae tick families (Cajimat et al., 2017; Suliman et al., 2017; Akuffo et al., 2016; Telmadarraiy et al., 2010; Ozdarendeli et al., 2008; Hoogstraal, 1979) such that many of the reported tick species were erroneously incriminated in vector and reservoir relationship with CCHFV based on a wrong interpretation of research data (Gargili et al., 2018). However, Hyalomma species are natural reservoirs with a vectorial capacity of transmitting CCHFV to humans due to their exophilic behavior (Garrison, 2019; Hoogstraal et al, 2019; Fajs, et al., 2014).

Biologically, Hylomma ticks can either survive as two and/ or three-host ticks, depending on prevailing habitat conditions. When the life cycle is completed among small mammals or ground birds, they tend to be two host ticks; and when large ruminants are involved, they complete it as three-host ticks (Walker et al, 2014). These ticks are biological hosts to CCHFV and the replicating viruses can be transmitted through transovarial or transstadial mechanisms. Horizontal transmission among ticks occurs if two ticks are feeding (a blood meal) adjacent to each other on a host (Telmadarraiy et al., 2010; Harry Hoogstraal, 1979). Infected larvae or

nymphs pass the infection to small animals (hares and hedgehogs and ground-dwelling birds), and in reciprocity, infected small animals are sources of infection to susceptible immature ticks. On the other hand, adult ticks spread the infection among large animals (Leblebicioglu, 2010; Walker et al., 2014).

The different species of the genus Hyalomma are widely distributed in Africa, Asia, and Europe (Spengler et al 2019; Walker et al, 2014; Leblebicioglu, 2010; Kaiser and Hoogstraal, 1963; Spengler and Bente, 2017) but not naturally found in America, Australia and the northern parts of Europe and Asia (beyond 50o N). H. marginatum, H. anotolicum, and H. asiaticum are associated with CCHF in Europe and Asia (https://www.cfsph.iastate.edu/Factsheets/pdfs/crimean_congo_hemorrhagic_fever.pdf, 2019; Leblebicioglu et al., 2015). H. dromedraii and H. truncutum, both have been reported imported from Africa to Australia and America, respectively (Barker et al, 2014; Mathison et al., 2015). Reports are also showing Hyalomma ticks emerging in the temperate regions like Sweden due to climate warming (Grandi et al., 2020). In Africa alone, over eleven Hyalomma species do exist, of which, H. truncatum covers most of the sub-Saharan region and H. rufipes spreads from the arid to rain forest climates of Africa. Another species common in the southern part of Africa is H. turanicum. The rest of the species are found in central, western and, most in the northern regions of Africa (Walker et al., 2014).

Berggren (1978) reported the presence of H. rufipes and H. truncatum in Malawi. H. truncatum was found to be present for the entire stretch of Lilongwe-Kasungu plain and some parts of Mzimba district; whereas H. rufipes was found, though scarcely, in the southern end of Lilongwe –Kasungu plain and also in Mwanza and Chinkhwawa districts. The seasonal activity of these two species Hyalomma varies and even within the species among the adults and the immature. Adults of H. rufipes are at peak during the early wet season and immature during the dry season. Whereas for H. truncatum, adults are numerous in late wet summer and the immature in autumn to spring months (Walker et al., 2014).

2.4 CLINICAL FEATURES AND PATHOGENESIS OF CCHF

CCHF is among the notorious hemorrhagic fever (VHF) diseases in humans having the widest global distribution. The clinical signs experienced in the early stages (pre-hemorrhagic) are non-specific: illness of sudden onset, fever, muscular pains, headache, vomiting, and abdominal pain. As the disease progress into a hemorrhagic stage, signs such as petechiae, purpura, epistaxis, hematemesis, hemoptysis, melena, and hematuria manifest. These signs are usually followed by multiple organ failure and endothelium collapse in the convalescence stage (WHO, 2018a; Balinandi et al., 2018; CDC, 2019). The incubation period varies from two to 14 days. Shorter incubation periods are observed following tick bites (WHO, 2018a).

The epithelium (skin and mucosa) of the host forms the first line of defense against the virus. Any discontinuity in the epithelium exposes the virus to the basolateral membrane which is adjacent to the endothelium of the peripheral blood vessels. A tick bite facilitates the delivery of the virus into the blood vessel or the basolateral membrane (Ergonul, 2012). CCHFV exposure through inhalation (Pshenichnaya and Nenadskaya, 2015) takes the virus to the alveolar epithelium. Two CCHFV pathological pathways in a susceptible host have been deduced. The first pathway is by causing direct cytopathic effects on parenchymal cells of the liver, spleen, and endothelial cells. The second pathway is to initiate the release of factors from the innate immune cells. (Arasli, 2016). After evading the epithelium, the virus either infects the endothelial cells or the locally available macrophages and dendritic cells in the basolateral membrane. These cells are supposed to release proinflammatory cytokines and chemokines but instead, there is suboptimal growth of these cells and failure to upregulate the antigen-presenting cells, hence there is no priming of the naive T-cell (Peyrefitte et al., 2010). The cellular immune cells cannot eliminate the virus from the host (Arasli, 2016); instead, they migrate, to regional lymph centers (local lymph nodes) and spleen, and thus the virus finds its way into the cardio-vesicular circulation. Injury to the spleen caused by the infection includes lymphoid apoptosis, splenocyte necrosis, and dilated sinusoids (Akıncı et al., 2015). Some virus finds their way directly into the circulatory system causing viremia. The virus is then disseminated through a hematogenous route to the liver and other organs (Arasli, 2016).

In the liver, the CCHFV induces hepato-necrosis by initiating endoplasmic reticulum stress and activation of both intrinsic and extrinsic pathways of hepatocyte apoptosis (Rodrigues et al., 2012). The clinical picture of increased liver injury associated enzymes: alanine aminotransferase (ALT) and aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine results in severe and fatal cases (Onguru et al., 2010).

The activated endothelial cell activation releases tumor necrotic factor, cytokine (IL-6 and IL-6) which are inflammatory mediators resulting in increased vesicular permeability and vasodilation. At the same time, platelet aggregation is activated and hence stimulation of both the intrinsic coagulation pathway. The coagulation mechanism is further disturbed by upregulating the coagulating factors whilst downregulating the anti-coagulating factors. On the other hand, infected macrophages release tissue factor that activates the extrinsic coagulation pathway (Arasli, 2016). Thus, both intrinsic and extrinsic, are activated, disseminated intravascular coagulopathy results. Coupled with the necrosis of the hepatocytes, phagocytosis, increased permeability of the endothelium, there is excessive hemorrhaging. The interaction of all these reactions may sum up to multiple organ failure (Esmon, 2004)

Infection of the cellular immune cells impairs the immune system through partial induction of dendritic cells and macrophages, delayed induction of interferons, apoptosis of lymphocytes causing lymphocytopenia, and weakening of the humoral immune system that paves way for farther multiplication of the virus (Arasli, 2016). The balance between the viral load and the host's immune system response becomes important in this case for the survival of the host. Patients with undetectable or very low levels of interferons or antibodies against CCHFV have a poor prognosis (Onguru et al., 2010).

2.5 DIAGNOSIS/ DETECTION OF CCHFV IN ANIMALS AND TICKS

2.5.1 DIAGNOSIS IN ANIMALS

Several tests /assays have since been developed for CCHFV and corresponding antibody detection in invertebrates, animals, and humans for clinical and epidemiological studies. These tests have made it possible for detection of immunoglobulins and antigens (for example

Enzyme-Linked Immunosorbent Assay (ELISA), immunofluorescence test (IF), and nucleic acids (like reverse transcriptase-polymerase chain reaction (RT-PCR) tests (Palomar et al., 2017; Mourya et al., 2014; Palomar et al., 2013; Tonbak et al., 2006).

Serological studies, using ELISA or IFA, have advanced the epidemiological understanding of both spatial and temporal distribution of CCHF. Thus, detection of IgM and IgG antibodies has enabled distinguishing of active/recent and old infections, respectively both in animals and humans (Mouryaet et al., 2012). Subsequently, it has been noted that IgM is short-lived in animals, three to seven weeks compared to three to five months in humans, post-infection (Burt et al., 1993) making animals better indicators for recent infections in a region/population. ELISA has been reported the most used and best assay in seroprevalence studies in animals (Spengler et al., 2016). Unfortunately, commercial testing kits specifically for animals were not available, until recently (Sas et al., 2018). All along, modified human immunological assays were used (Schuster et al., 2016; Mertens et al., 2015). Successful application of these serological tools requires the timely collection of samples as levels and classes of antibodies tend to vary greatly with the stage of disease/infection progression.

Nucleic acid amplification tests (NAAT), a group of molecular diagnostic methods, especially RT-PCR, have led to genetic characterization and evolutionary studies of CCHFV which generates valuable and reliable information for molecular epidemiology. Furthermore, monitoring of the spatial distribution of different strains (clades) across the globe has been made possible (Cajimat et al., 2017; Chinikar et al., 2016; Palomar et al., 2013; Aradaib et al., 2011). Phylogenetic analysis has also helped to trace the existence of the virus way beyond its first clinical description through evolutionary analysis. Although popular in the studies of CCHFV, RT-PCR suffers limitations in detection due to genotypic variations among CCHFV variants. This calls for a thorough understanding of the geographical distribution of CCHFV genotypes and the use of appropriate primers/probes, based on the expected virus in a particular region. The increased emergence of CCHFV in novel regions makes the situation complex, though. Just like the serological tools, the sampling time is critical since viruses can only be detected during the viremic period, which is a short time interval in animals (Sorvillo et al., 2020). Unlike serological tests, RT-PCR coupled with downstream techniques like sequencing and phylogenetics, have been instrumental in molecular epidemiology, and

evolutionary studies (Anagnostou and Papa, 2009) among other applied sciences.

Many other tests/assays are available, like complement fixation test (CFT), immunodiffusion (ID), and reverse passive hemagglutination (RPHA); however, their use is mostly for research purposes than diagnosis. Cell cultures and animal models have been used especially for CCHFV isolation (Shepherd et al., 1988) in biosafety level four (BSL 4) facilities. Even though the use of laboratory animal models has made it possible to amass CCHF pathophysiology knowledge, the application has been limited by the scarcity of BSL 4 facilities (Garrison, 2019; Mazzola and Kelly-Cirino, 2019).

2.5.2 DIAGNOSIS IN TICKS

CCHFV have been detected in over 31 species of ticks (Moraga-Fernández et al., 2020; Schulz et al., 2020; Hoogstraal and Read, 2019). Longer persistence of CCHFV in ticks allows ease of detection while the chances of detection are reduced in animals due to a short viremic period. Assays targeting the CCHFV genome or specific virion proteins as described in animal diagnosis are used in ticks as well (Schulz et al., 2020; Yesilbag et al., 2013). In the recent past, RT-PCR and real time- PCR have been used extensively (Moraga-Fernández et al., 2020; Drosten et al., 2003).

2.5.3 DIFFERENTIAL DIAGNOSIS OF CCHF

Many diseases in humans manifest similar signs as CCHF, especially in the initial stages of the disease, hence difficult to distinguish it from other diseases such as malaria and typhoid fever which are endemic in the sub-Saharan African region. Other diseases include leptospirosis, rickettsial infection, and meningococcemia (Appannanavar and Mishra, 2011). CCHF needs to be differentiated from other viral hemorrhagic fevers including Ebola cases in Africa (WHO, 2018).

2.6 TREATMENT, PREVENTION, AND CONTROL OF CCHF

As an occupation hazard, CCHF prevention information is invaluable to high-risk groups. Risk reduction can be through limiting the contact of humans and ticks and also ticks and animals. Both chemical and physical methods are available such as the use of tick repellents, use of protective clothing (long sleeve and pants), visual inspection, and appropriate removal of ticks. The use of acaricides on livestock reduces the transmission of virus between animals and ticks, thus eliminating the amplification factor of the virus. Avoidance of direct contact with animal body fluids from viremic animals reduces the risk among veterinarians and abattoir workers (Sorvillo et al., 2020; Tipih and Burt, 2020; Balinandi et al., 2018; Hawman and Feldmann, 2018). In hospital setups, barrier nursing is the most efficient risk reduction approach (WHO, 2018a).

Since the discovery of CCHF, efforts to develop a vaccine for CCHF have been intense but up to date, no vaccine has been commercialized (Shayan et al., 2015). In a comprehensive review, Tipih and Burt, (2020) discussed a collection of CCHF vaccine candidates still under development ranging from subunit vaccines, plant-based vaccines, virus-like replicon, virus-like replicon particle, mRNA vaccines, viral vectorial vaccines to inactivated vaccines. Common challenges faced in all these endeavors include lack of safety trials, CCHFV genetic diversity, lack of delineation of correlating CCHF protection factors in model animals, and lastly lack of efficacy trials (Sorvillo et al., 2020). Bulgaria has been using, locally though, chloroform-based inactivated vaccine since 1974, but due to lack of safety (due to crude production procedures) and efficacy data; this vaccine has not been approved and commercialized to date (Shayan et al., 2015). The genetic diversity within CCHFV strains poses a great challenge in the possibility of a universal vaccine such that more studies of local strains in circulation per foci are a priority. Although most of the current candidate vaccines have shown a potential to elicit an immune response (cellular or humoral) in model animals, they all fail to confer protective immunity upon lethal dose infection challenge (Sorvillo et al., 2020; Tipih and Burt, 2020; Dowall et al., 2015).

Despite the wide use of ribavirin drugs in managing human clinical cases, there remains no approved treatment for CCHF in humans nor animals (Al-Abri et al., 2017). Al-Abri et al.,

further reviewed that studies that reported the use of ribavirin most had small samples and lacked placebo controls, hence efficacy of ribavirin against ribavirin efficacy in use against CCFH remains not clear.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY AREA

The study was carried out in Malawi, a landlocked and agriculture-based country covering 118 484 square kilometers in sub-Saharan Africa, situated within latitudes 9° and 18°S, and longitudes 32° to 36° E. She shares borders with Tanzania to the north, Mozambique to the southeast, and southwest, and Zambia to the west. Approximately 17.5 million people (NSO, 2018) occupy her land and own 1,763,704 cattle, 9,147,837 goats, and 331,272 sheep (DAHLD, 2019) among other livestock species, largely raised communally.

The country is portioned into eight Agricultural Development Divisions (ADDs). A division is made up of districts and each district is divided into Extension Planning Areas (EPAs), which are further subdivided into veterinary stations. This study was conducted in two ADDs, Kasungu and Lilongwe ADDs, involving seven districts. The two ADDs are both located centrally in the country and largely in the Lilongwe-Kasungu plain. The plain has a savanna tropical climate and experiences hot dry summer (September to November), hot wet summer (December to March; rainfall range: 801 to 1000 mm per annum) and moderately cold winter (April to August) seasons (Vincent et al., 2014). Livestock is grazed together on communal grasslands during daytime and housed within villages at night. During post-harvest time, animals are grazed in crop fields; and in the crop season, animals are grazed in dambos and uplands. Maize is the main food crop with tobacco, groundnuts, and soya beans being common cash crops grown in this plain (FAO, 2009). The plain has experienced deforestation and clearing of marginal lands as a consequence of poor farming practices (Munthali and Murayama, 2013). This plain was reported to be infested with Hyalomma ticks (Berggren, 1978). Hence, it was found appropriate to conduct this study in this region.

3.2 STUDY DESIGN AND SAMPLE COLLECTION

3.2.1 STUDY DESIGN AND SAMPLE SIZE DETERMINATION

A cross-sectional study was conducted in April 2020, in seven districts; three in Lilongwe and four in Kasungu ADDs. The sample size was determined using Emergency Nutrition

Assessment (ENA for SMART, 2011) software. The seroprevalence of CCHF in cattle had never been estimated before in Malawi. Hence, a 50% seroprevalence (p) was used in the calculations for sample size. Further, 6% relative precision (d), 95% confidence level (Z(0.95)), and design effect of 1.5 (Dohoo et al., 2004) were used to estimate the sample size. Using these parameters, a sample size of 436 cattle was determined.

A multistage sampling approach was taken where Extension Planning Areas (EPA) sampled randomly in each district and sample collection centers (village trading centers and/or veterinary stations) considered as clusters within these EPAs. The minimum number of animals per district was allocated proportionally based on district cattle populations. Figure 3-1 below shows the number of cattle sampled per district. Sample collection centers were randomly identified in respective EPAs within districts, mostly village trading centers and/or veterinary stations. Super herds, defined as cattle belonging to different farmers but managed as a single herd (Munyeme et al., 2010), did exist in the study area; hence, for this study, a herd was defined as all cattle groupings under one management custody. Cattle farmer registers at sample collection centers were used as sampling frames, such that cattle herds were selected using a systematic random sampling technique. Assistant veterinary officers were consulted for estimation of average herd size and an average of ten cattle per herd was suggested and hence used for calculating the number of herds to be sampled per particular sampling center. The sampling interval for herds at a particular center was calculated by dividing the total number of herds by the number of herds required per that center. Individual animals within herds were assigned arbitrary identification numbers which were later used for simple random selection by rough draw. The study included cattle of all ages, sex, and breeds that were raised communally in the study area. However, the study excluded pregnant cows in second and third trimesters and clinically ill cattle.

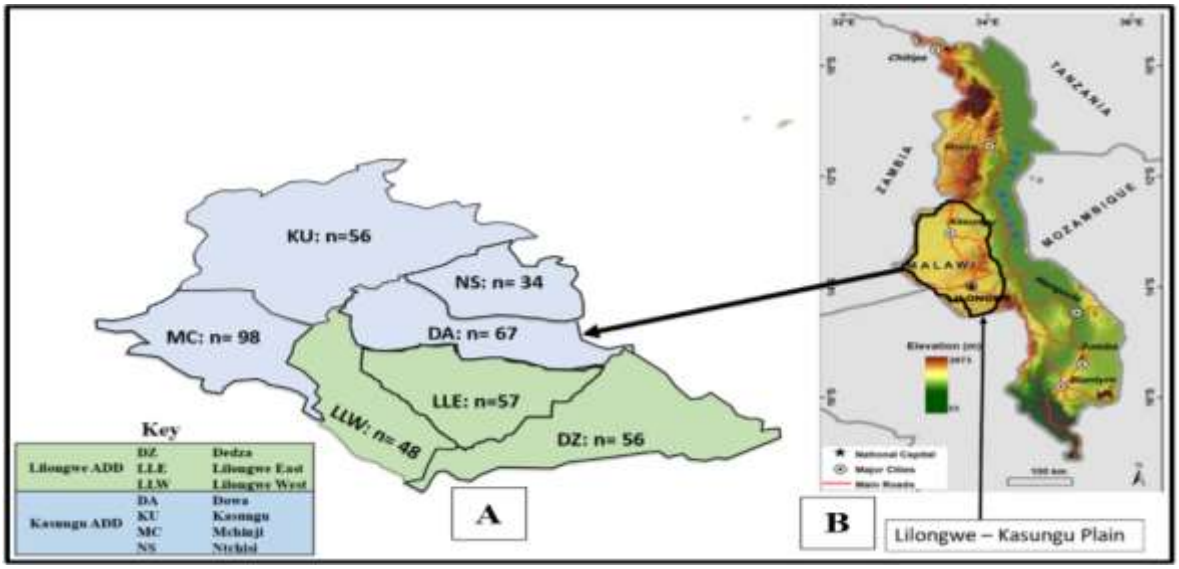

Figure 3-1: Geographical location of the study area. [A] Map showing the seven study districts and their positions in the agricultural divisions (Lilongwe and Kasungu). (n = number of animals sampled per particular district). [B] Map of Malawi (Li et al., 2017) showing the location of the study area in the country.

3.2.2 QUESTIONNAIRE ADMINISTRATION

Cattle farmers were interviewed using a structured questionnaire which was administered by the principal investigator face to face. The questionnaire had three parts: the first part gathered information about the demographics of cattle farmers (age, sex, marital, status, and education level). The second part obtained information about cattle herd dynamics and management. The herds were categorized as (small (1-6 animals), medium (7-13 animals), and large (>14). The source of cattle was recorded as within district = all cattle in a herd sourced from within the district; outside district = at least one cattle sourced from outside the district). The ticks on cattle were recorded as present or absent. Whether a farmer practiced tick control measures or not, it was recorded as present or absent. Tick control methods were recorded as per their names i.e. spraying, dipping and other (pour/spot-on, injection, removing with hands or none). The Type of grazing land was categorized as dambo, upland, or both (dambo and upland). Selected cattle owners who did not consent for themselves and their herds, were replaced by other cattle farmers from the same sample collection centers.

3.2.3 CATTLE BIOLOGICAL DATA

The age and sex of the cattle were determined by physical inspection. Animal age was inquired from the respective cattle farmer and verified by dentition method of age estimation (Torell et al., 2003; Mushonga et al., 2020) and recorded in years. Age was converted from continuous to categorical data with the following levels: 1-12 months, 13-24 months, 25-48 months, and >48 months. These categories were arrived at based on assumed cattle cumulative frequency of exposure to ticks; with young animals being less exposed and old animals being repeatedly exposed (Simuunza et al., 2011). Animal sex was recorded as either male or female.

3.2.4 SERA SAMPLE COLLECTION

Cattle were bled aseptically in plain vacutainer tubes (five ml per animal) using jugular or coccygeal venipuncture approaches. Each tube was labeled according to the district, sample collection center, herd number, and animal number. The blood samples were stored on ice (≈ 4°C) in cooler boxes. Serum was separated from the collected blood within 36 hours from the time of collection at Central Veterinary Laboratory (CVL) in Lilongwe. The serum separation was done by centrifugation at 1000 xg for 15 minutes as per World Organization for Animal Health (OIE, 2008) protocol and later aliquoted into two milliliters Eppendorf tubes.

3.2.5 TICK COLLECTION

Ticks were collected from animal body surfaces by handpicking. The sampled ticks were collected in 50 mL falcon tubes with perforated lids for easy ventilation. Fresh pieces of grass/leaves were added to each tube to maintain good ventilation and humidity. Each tube was labeled according to the district, collection center, herd identity, and date of tick collection. Ticks were incubated at 18°C at CVL in Lilongwe while waiting for identification. Ticks were identified using morphological features (Walker, et al., 2014) and only those that were alive at the time of identification were kept for RNA extraction. Ticks belonging to a particular herd and species, sequentially, were grouped and put in a two-milliliter Eppendorf tube.

3.2.6 SAMPLE STORAGE AND TRANSPORTATION

Both samples (sera and ticks) were immediately stored at -80°C, at African Union Centre for Tick- and Tick-Borne Diseases (AU-CTTBD). Samples were triple packed and transported by road, in a frozen state, to the Disease Control Department laboratories in the School of Veterinary Medicine of the University of Zambia (UNZA) in August 2020. At UNZA, the samples were stored at -20°C for three months after which laboratory analysis was conducted.

3.3 LABORATORY SAMPLE ANALYSIS

3.3.1 ELISA PROTOCOL

ID Screen® CCHF Double Antigen Multi-species (IDvet; Garbles, France) sandwich ELISA was used to screen both IgG and IgM specific antibodies against CCHFV (Sas et al., 2018) in serum samples. Ninety-six well ELISA microplates were used and wells of each plate were partitioned as follows: A1 and B1 wells for substrate control, C1 and D1 for conjugate control, E1 and F1 for the negative control, and lastly, G1 and H1 for positive control. About 50µL of the prepared wash solution was dispersed to all the wells using a multi-channel pipette. Then, 30µL of the negative control was added to each of the designated E1 and F1 wells. Test serum samples in duplicates were added to the remaining wells. Positive controls were loaded after the samples to avoid contamination. Figure 3-2 below shows the ELISA plate map for samples and sets of controls. The plates were covered, genteelly mixed, and then incubated at 25oC for 45 minutes on the bench. After incubating, all the wells were washed five times with 300µL of wash solution per wash per well. Thereafter, 50µL of the conjugate was dispersed to each well except wells A1 and A2. The plate was then covered with plastic and incubated at room temperature for 30 minutes. The excess conjugate was removed by washing five times with 300µL wash buffer per well per wash. After removing the excess conjugate, 100µL of substrate solution was added to each well except wells C1 and D1. The plate was then covered and incubated in dark at room temperature for 15 minutes. The substrate reaction was stopped by adding 100µL of stop solution, in the order of substrate addition to avoiding variation in substrate reaction time. The optic density was read and recorded at 450nm as per the manufacturer's instruction.

	1 (Controls)	2	3	4	5	6	7	8	9	10	11	12
A	Substrate	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
B	Substrate	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
C	Conjugate	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
D	Conjugate	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
E	Negative	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
F	Negative	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
G	Positive	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
H	Positive	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*

Figure 3-2: Allocation of controls and test samples to the ELISA microplate wells

NB: Test S= test sample, d= duplicate of the test sample in the row above it.

Validation of the protocol: The test was valid if the mean optic density of the positive control (ODpc) was greater than 0.350, and the ratio of the mean ODpc to mean optic density for negative control (ODnc) was greater than three (Table 3-1). Interpretations of the test ODs were based on the ratio of the mean sample optic density to ODpc, expressed as a percentage (S/p *100). Samples with S/p percent less or equal to 30% were considered negative and samples with S/p percent greater than 30% were considered positive. Table 3-1 below shows validation data for the ten plates that were run and all the runs were valid on both criteria.

Table 3-1: Optic densities (OD) for positive and negative controls and their calculated ratios

Plate #	Mean ODpc	Mean ODnc	ODpc/ODnc ratio
1	0.799	0.046	17.549
2	0.924	0.052	17.930
3	0.999	0.045	22.200
4	0.888	0.046	19.516
5	0.656	0.052	12.615
6	1.029	0.049	21.206
7	0.888	0.048	18.489
8	1.008	0.047	21.666
9	0.908	0.056	16.360
10	1.456	0.047	30.968

NB: All mean ODpc were valid when > 0.350 and all ODpc/ODnc were valid when >3

3.3.2 RNA EXTRACTION FROM TICKS

Individual ticks were washed in phosphate-buffered saline (PBS) solution, 70% ethanol, and PBS, sequentially. Dulbecco's Modified Eagle Media (DMEM) was used as a homogenizing media where 500µL of DMEM was dispensed in 2mL screw cap smashing tubes containing two 2mm diameter beads. An electric micro smasher (Tomy-MeDICO LTD, Japan), set at 3500 rpm for 30 seconds, three runs for each tick were used. RNA was then extracted from 140 µL of the homogenate per sample using QIAGEN RNAeasy kit (QIAGEN, Hilden, Germany) by using Buffer AVL -Carrier RNA mixture for cell lysis and precipitation of the freed RNA material. QIAmp spin columns were used to filter cellular particles by centrifuging at high revolution (8,000 -14,000 rpm) at 4°C. Absolute ethanol (100% ethanol) and provided wash solutions (AW 1 and AW 2) were used to emulsify and clean the extract. RNA was eluted in 60µL AVE buffer (RNase free water with 0.04% sodium azide (NaN3) and was immediately stored at -80°C until required for use. The whole protocol followed the manufacturer's instructions.

3.2.2 NESTED RT-PCR PROTOCOL

A nested RT-PCR design was used. First-round used one-step RT-PCR with CCHFV R2/F3 primers followed by a second round where a conventional PCR with CCHFV R3/F2 primers (Rodriguez et al., 1997) were used by using the one-step RT-PCR products as templates.

The QIAGEN One-Step RT-PCR kit (Qiagen, Hilden, Germany) was used for the synthesis of complementary DNA (cDNA) and PCR. The protocol and associated reagents used in this procedure were adopted from QIAGEN® OneStep RT-PCR Handbook (2012). For every reaction, 1.5µL of the template RNA, 4.0µL of 5x QIAGEN One-Step RT-PCR Buffer, 0.8µL of dNTP Mix, 11.9µL of 5x Q-Solution, 0.5µL of R3 primer (5'-GACAAATTCCCTGCACCA-3'), 0.5µL of F2 primer (5'-TGGACACCTTCACAAACTC-3'), 0.8µL QIAGEN One-Step RT-PCR Enzyme Mix was used. The total volume was 20µL. All the reaction mixtures were prepared on ice. A master mix was prepared by mixing (after thawing) all the reagents, with exception of the template RNA. The master mix was thoroughly mixed by vortexing and spinning before being dispensed into the PCR tubes. For every sample, 1.5 µL of template RNA was added to a PCR tube containing 18.5 µL of the

master mix. All steps of the protocol up to this step were performed on ice (4oC). The temperature profile for thermal cycler was set as follows: Reverse transcriptase reaction at 50oC for 30 minutes and denaturation at 95oC for15 minutes; followed by amplification reactions of 45 cycles with denaturation (94oC for 60 sec); annealing (50oC for 30 sec) and the extension (72oC one minute). The final reaction was an extension at 72oC for 10 minutes. PCR products were stored at 4oC in waiting for gel electrophoresis.

The PCR products were visualized using 1.5 % agarose gel prepared by dissolving agarose powder (Cleaver Scientific Ltd, Rugby, UK) in TAE buffer (Tris-base 2M, Acetic acid 2M, 0.005M EDTA, and distilled water) in a ratio of 1.5:1 (mass (g) /volume (mL)) and colored with a drop of ethidium bromide. Five microliters of each sample PCR product were mixed by pipetting with a drop of loading dye, and loaded onto the electrophoresis gel and later in the electrophoresis chamber and run for 30 minutes. A 100bp ladder/ marker was used in a Benchtop 3UV transilluminator (BioDoc-it Imaging system UVP, CA, U.S.A)

The conventional PCR was performed using Ex-Taq enzyme and CCHFV F3/R2 set of primers targeting 260bp long region of the S segment sequence. The following reagents and volumes were used for each reaction: 37.5µL of nuclease-free water, 5.0µL of 10X buffer, 4µL of dNTPs, 1.0µL of Forward Primer (CCHFV F3) 290 5`>GAATGTGCATGGGTTAGCTC<3` 309nt position, 1.0µL of Reverse Primer (CCHFV R2) 549 5`>GACATCACAATTTCACCAGG>3`530 nt position, 0.25µL of an ex-Taq enzyme, and 1.0 µL of DNA template (the one-step RT-PCR product). The total volume was 50 µL. The programing of the thermal cycler was as follows: Initial denaturation at 92oC for two minutes. This was followed by 35 cycles of amplification as follows: denaturation (94°C for 30 sec), annealing (at 50°C for 60 sec), and extension (72°C for two min). The final extension was set at 72°C for 10 minutes. The order of preparing the master mix and adding the samples and as well as visualizing PCR products was the same as described for One-step PCR above.

3.3 DATA ANALYSIS

Data was collected through the administering of a structured questionnaire, observing herd/cattle attributes, and laboratory analysis of blood and tick samples. All data were entered, cleaned, and validated in Microsoft™ excel spreadsheets. All descriptive and inferential

analysis were performed in IBM SPSS version 20 (IBM Corp) and Microsoft™ excel spreadsheets.

The survey summaries and univariate analysis involved largely calculating counts, means, frequencies, and relative frequencies. The CCHFV ELISA test results (positive or negative) were the dependent variable in this study and the following were independent variables (risk factors): cattle age, cattle sex, cattle management, and ecological factors. Bivariate analysis and testing related hypothesis was performed using Pearson Chi-Square test of association (and Fisher's exact test, where appropriate) to test for association between CCHFV seropositivity and the independent variables. All independent variables that were found to be associated with CCHFV seropositivity at $p \leq 0.250$, were included in binary logistic regression model analysis. Hosmer-Lemeshow test and Omnibus test for model coefficients were used to determine the fitness of the model and its significance, respectively. All statistics were considered significant at $p \leq 0.050$. Display of data about geographical distribution was done using Microsoft™ PowerPoint editable maps (https://www.premiumslides.com/en/malawi-maptemplate-editable-powerpoint.html#).

3.4 ETHICAL CONSIDERATIONS

The protocol used in this study was reviewed and approved by the Animal Health Committee (AHC) of the Department of Animal Health and Livestock Development (DAHLD) Lilongwe, Malawi (letter number: AHC/01/2020) for field data and sample collection. Biomedical Research Ethical Committee (REC) of the University of Zambia (UNZA), Lusaka, Zambia (letter number: Ref. 780-2020) cleared the study for academic and laboratory work. Before sample collection and questionnaire administration, cattle farmers were consented in writing for those who could write and verbally for those who could not write. Animal safety and welfare were ensured by using appropriate restraining methods (Barley rope or halter methods) and observance of aseptic techniques when collecting blood. During the exercise, primary animal health care (i.e., wound treatment, deworming, and tick-borne disease treatment) was provided to some animals, where necessary, by the researcher. Samples were imported into Zambia with authorization from the Department of Veterinary Services of the Ministry of Livestock and Fisheries, Lusaka, Zambia (import permit number 56/2020(VTHQ/8/3/18).

CHAPTER FOUR

4.0 RESULTS

4.1 DESCRIPTIVE STATISTICS OF THE STUDY POPULATIONS

The questionnaire was administered to 109 cattle farmers (104 males and 5 females). The farmers had a median age of 50 years (minimum 20 years and maximum 87 years). Of these 7.3% (8/109) had no formal education, 61.5% (67/109) primary education, 27.5% (30/109) secondary education and 3.7% (4/109) tertiary education. All cattle farmers knew ticks. About 62.4% (68/109) considered ticks to be very important to cattle health, 27.5% (30/109) as less important to cattle health, and 10.1% (11/109) did not associate ticks with the health of cattle. Of the interviewed cattle farmers, 61.5% (67/109) controlled ticks in their respective cattle herds.

Out of the 436 calculated sample size, 416 cattle were sampled, representing 95.4% (416/436) sample collection success. Some cattle herds could not be reached because of poor roads. Figure 4-1 below shows details of the description of the study cattle population structure. The herd structure had more cattle aged more than 25-48 months and mostly in small herds.

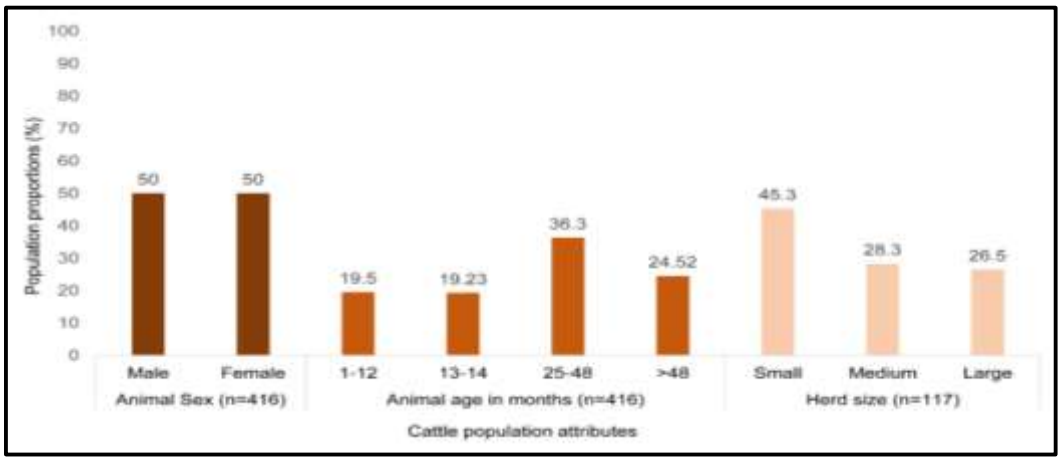

Figure 4-1: Study cattle population structure

Variations were observed in the way traditional cattle herds were managed in the study population (Table 4-1). Many cattle herds (90.59%; 106/117) were infested with ticks and tick control was reported to be practiced in 62.04% (67/117) of the herds.

Table 4-1: Distribution of cattle herds across different cattle management forms

Factor	Category	Number of herds	Proportion (%)
Grazing land type	Dambo	94	80.34
	Upland	11	9.40
	Both (dambo and upland)	12	10.26
Tick control	Present	67	62.04
	Absent	41	37.96
Presence of ticks in the herd	Present	106	90.59
	Absent	11	9.40
Tick control frequency	None	41	37.60
	Whenever necessary	33	30.30
	Monthly	19	17.40
	fortnightly	11	10.10
	Weekly	4	3.70
Method of tick control	No tick control	41	37.60
	Spraying	56	51.40
	Dipping	1	0.90
	Mixed methods	11	10.10

The following five tick species were collected and identified: Amblyomma variegetum, Rhipicephalus Boophilus decoloratus, Rhipicephalus Boophilus microplus; Hyalomma truncatum, and Rhipicephalus appendiculatus (Figure 4-2). Rhipicephalus Boophilus decoloratus was the most prevalent among the tick species in six of the study districts. H. truncatum was found in five of the seven districts with the highest herd prevalence (69%) observed in Dowa district whilst none was observed in Lilongwe West and Dedza districts. Rhipicephalus appendiculatus was the least infesting tick species.

Table 4-2: Tick species distribution by district at cattle herd level in Lilongwe and Kasungu ADDs

District		Tick species									
		A. var.		Rh. (B) dec.		Rh. (B) mic.		H. tru.		Rh. app.	
	herds sampled	herds %	Av. # ticks/herd	herds %	Av. # ticks/herd	herd %	Av. # ticks/herd	herds %	Av. # ticks/herd	herds %	Av. # ticks/herd
LLE	34	18	0.35	68	6.62	0	0.00	9	0.12	3	0.03
LLW	15	47	0.07	6	8.60	15	0.07	0	0.00	13	0.07
DZ	18	22	0.44	89	6.50	0	0.00	0	0.00	0	0.00
KU	13	54	3.23	69	4.54	0	1.15	23	2.08	0	0.00
NS	7	57	4.57	71	9.86	14	0.29	57	2.29	0	0.00
DA	13	69	2.38	85	9.46	31	0.62	69	1.77	8	0.15
MC	17	47	1.29	100	14.47	12	0.00	6	0.12	0	0.00
Overall	117		1.26		8.27		0.22		0.62		0.03

Abbreviations: LLE= Lilongwe East; LLW= Lilongwe west; DZ=Dedza; KU= Kasungu; NS= Ntchisi;

(DA= Dowa and MC= Mchinji.

Abbreviations: A.var. = Amblyomma variegetum; Rh (B) dec.= Rhipicephalus Boophilus decoloratus; Rh. B. mic. = Rhipicephalus Boophilus microplus; H. tru. = Hyalomma truncatum; Rh. App. = Rhipicephalus appendiculatus)

Optic densities for serum samples were determined and the ratios of serum sample OD to positive control OD, expressed as percentages were calculated for all the samples (Figure 43).

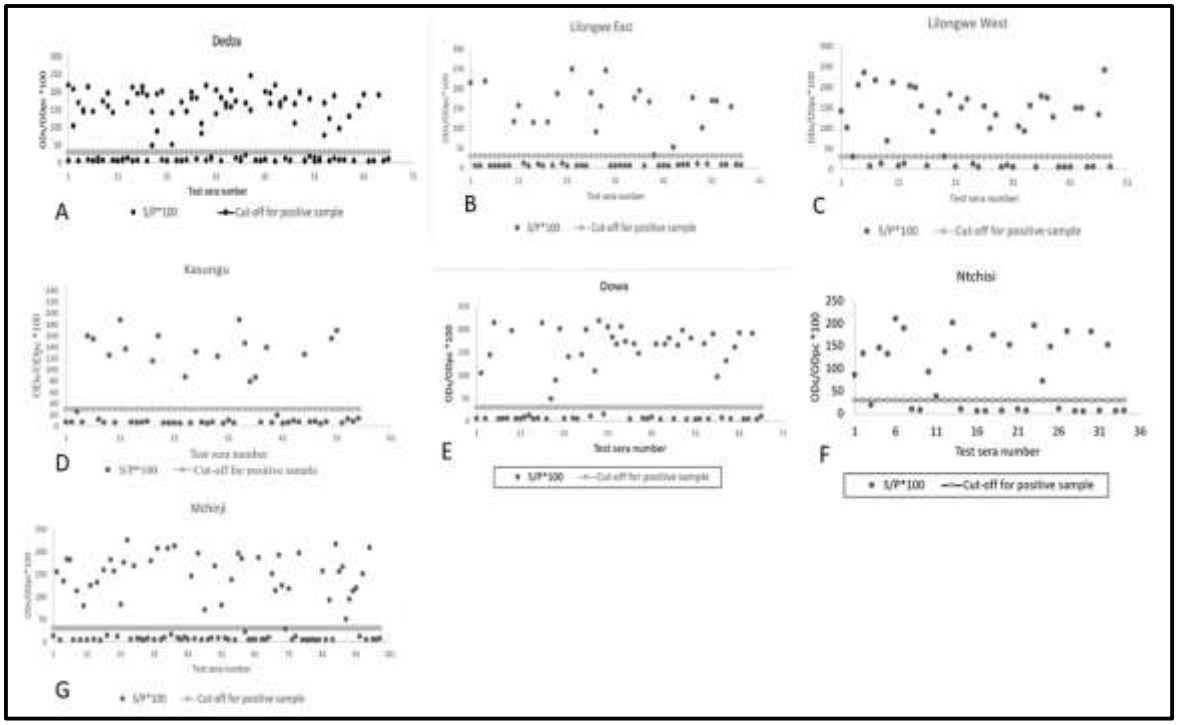

Figure 4-2: Ratios of Optic densities (OD) for sample (S) to Optic density for the positive control (PC) in percentages used to determine positivity/ negativity of a serum sample (S/P*100). A cut-off point of 30% is shown by grey dots with a trend line. All study districts individual sera data are shown in charts A-G.

4.2 SEROPREVALENCE OF CCHFV SPECIFIC ANTIBODIES AT INDIVIDUAL CATTLE LEVEL

The overall seroprevalence of CCHFV specific antibodies at individual cattle was estimated to be 46.9% (n = 416; 95% CI: 42.02-51.82). The seroprevalence in Lilongwe ADD was 50.3% (n= 161; 95% CI: 42.33-58.28%) and in Kasungu ADD was seroprevalence was 44.7% (n=255; 95% CI: 38.5-51.04%). The CCHFV seroprevalence differed among the study districts (Figure 4-3).

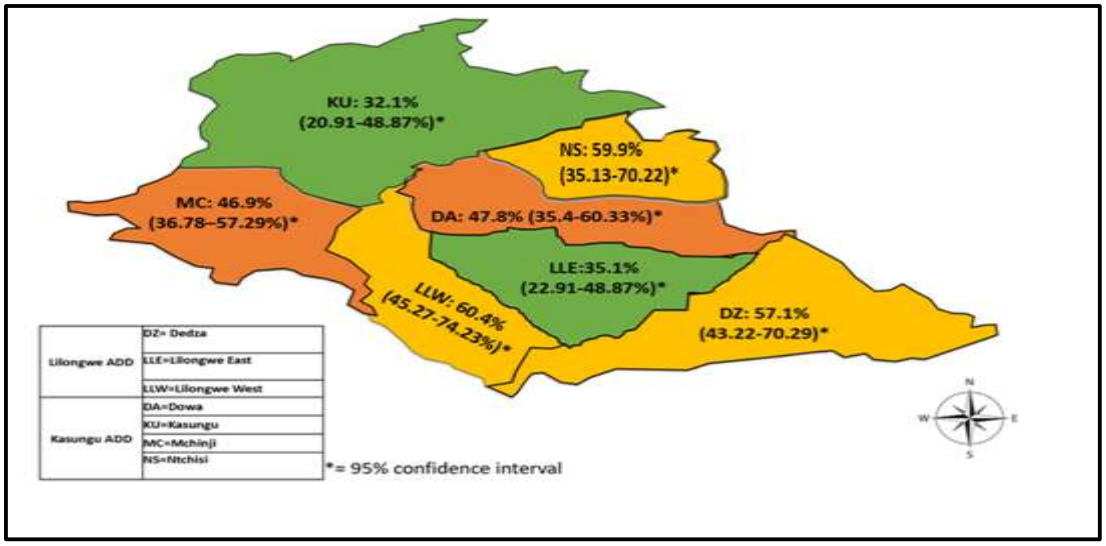

Figure 4-3: Spatial distribution of seropositivity to CCHFV in cattle in the study area

Bivariate analysis (Pearson Chi-square test of association) showed a significant association between the dependent variable (CCHFV specific antibodies) and the biological risk factors (cattle age and sex) (Table 4-3).

Table 4-1: Bivariate analysis of association for biological risk factors to seropositivity at individual cattle level

Risk factor	Category	n	Seroprevalence (%)	95% C I	p-value
Sex	Male	208	36.5	29.99-43.48	<0.001
	Female	208	57.2	50.18-64.03	
Age	1-12	83	25.3	16.39-36.04	<0.001
	13-24	80	31.3	21.35-42.59	
	25-48	151	58.3	49..98-66.24	
	>48	102	59.8	49.63-69.39	

n = number of cattle; CI= Confidence interval

CCHFV seropositivity was also significantly associated with district (location) (p-value = 0.025), tick presence on cattle (p-value = 0.016), grazing land type (p-value = 0.013), source of animal (p-value 0.24) and herd size (p-value = 0.21) (Table 4-4).

Table 4-2: Bivariate analysis of test of association for CCHFV antibodies and associated risk

Risk factor	Category	n	Seroprevalence (%)	95% CI	p-value
District	Dedza	56	57.1	43.22-70.29	0.025**
	Dowa	67	47.8	35.40-60.33	
	Kasungu	56	32.1	20.29-45.96	
	Lilongwe East	57	35.1	22.91-48.87	
	Lilongwe West	48	60.4	45.27-74.23	
	Mchinji	98	46.9	36.78-57.29	
	Ntchisi	34	59.9	35.13-70.22	
Tick presence in the herd	Present	384	48.7	43.6-53.82	0.016**
	Absent	32	25.0	11.46-43.4	
Grazing land type	Dambo	326	44.8	39.3-50.36	0.013**
	Dambo and upland	40	40.0	24.86-56.67	
	Upland	50	33.0	51.23-78.79	
Tick control	Present	254	46.9	40.59-53.19	0.854
	Absent	133	45.9	37.22-54-72	
Animal source	Within district	331	57.7	42.24-53.27	0.241**
	Outside district	56	39.3	26.5-53.25	
Presence of other stocks	Present	383	46.7	41.65-51.87	0.336

	Absent	4	25.0	0.063-80.59	
Herd size	Small	127	52.0	42.93-60.91	0.21**
	Medium	113	48.7	39.16-58.26	
	Large	176	42.05	34.66-49.7	
Significance of ticks to animal heath	Not important	10	80.0	55.5-99.7	0.72
	Important	30	70.0	50.6-85.27	
	Very important	68	67.65	55.21-78.49	

factors at individual cattle level

n = number of subjects involved; CI= Confidence interval; ** statistically significant at p-value ≤ 0.25

Maximum likelihood estimates of the predictors of the cattle being seropositive to CCHFV were calculated as shown in Table 4-5 below. All variables with p-values ≤ 0.250 in the bivariate analysis were included in the model. The Hosmer-Lemeshow test was not significant (p-value = 0.873) and the Omnibus test for model coefficients was significant (p-value < 0.001). The results indicated that district (cattle locality), cattle age, cattle sex, presence of ticks on cattle, and type of land where the cattle were grazed were significant predictors of cattle being CCHF seropositive.

Table 4-3: Maximum likelihood estimates of factors associated with cattle being CCHF seropositive

Risk factor	Category	OR	95% CI	p-value
District	Mchinji	r		
	Dedza	2.186	0.985-4.849	0.054
	Dowa	0.654	0.268-1.556	0.329
	Kasungu	0.723	0.336-1.558	0.408
	Lilongwe East	1.167	0.526-2.589	0.704
	Lilongwe West	2.741	1.191-6.307	0.018***
	Ntchisi	5.005	1.383-18.111	0.014***
Animal Age	1-12 months	r		
	13-24 months	1.257	0.587-2.689	0.556
	25-48 months	4.33	2.196-8.537	<0.001***
	>48	4.229	2.032-8.798	<0.001***
Animal Sex	Male	r		
	Female	2.478	1.568-3.944	<0.001***
Presence of ticks in the herd	Absent	r		
	Present	3.206	1.208-8.509	0.019***
Grazing land type	Dambo	r		
	Both (Dambo and Upland)	0.530	0.182-1.542	0.244
	Upland	4.489	1.799-11.200	0.001***

*** Statistically significant at $p \leq 0.05$. Abbreviation: OR= Odds ratio, CI= Confidence interval, r = reference category

Cattle aged 25-48 months and older than 48 months were four times more likely to be seropositive to CCHFV (OR: 4.33; 95% CI: 2.196-8.537 and OR: 4.229; 95% CI: 2.032-8.798, respectively) compared to those 1-12 months old cattle. Female cattle were two times more likely to be CCHFV seropositive compared to male animals (OR: 2.478; 95% CI: 1.568-3.944). The presence of ticks on cattle increased the likelihood of being seropositive for CCHF by three times (OR: 3.206; 95% CI: 1.208-8.509). The likelihood of being seropositive for CCHF was 4.5 times more in cattle grazing in uplands than those grazing in dambo lands (OR: 4.489; 95% CI: 1.799-11.2).

The mean predicted probability of being seropositive to CCHF was calculated and plotted against each of the variables that were significant in the model (Figures 4-4 to 4-8).

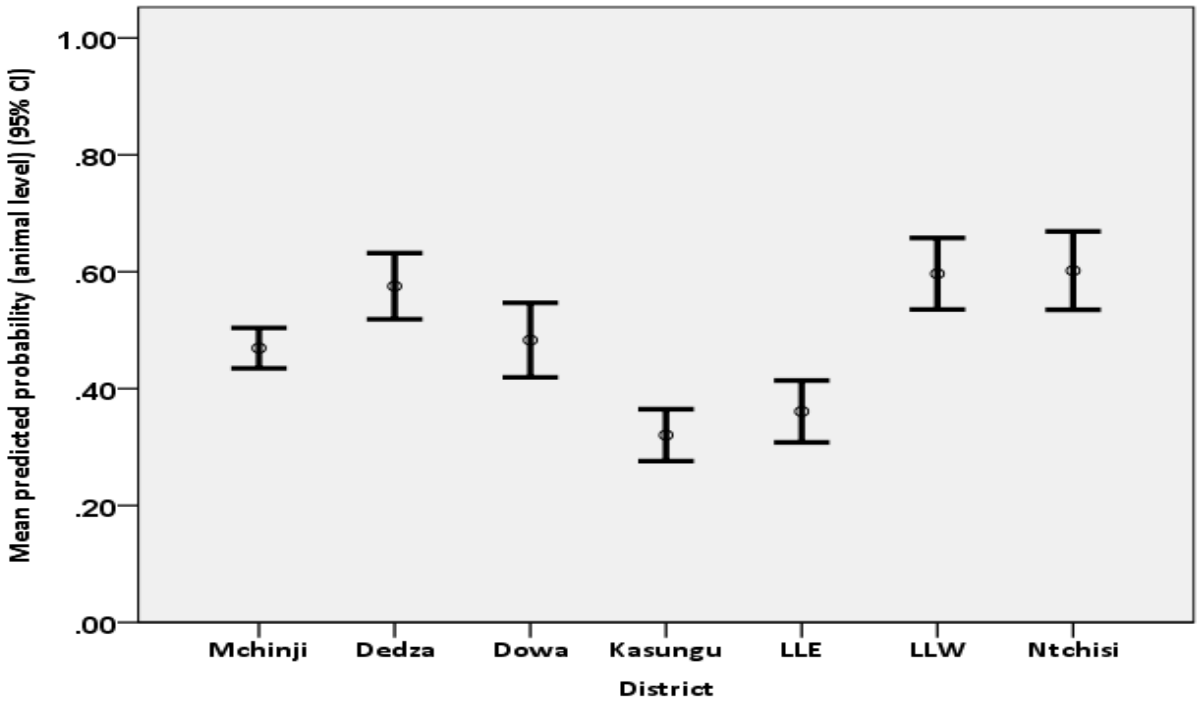

Figure 4-4: Mean predicted probability of CCHFV seropositivity for each district in the study area

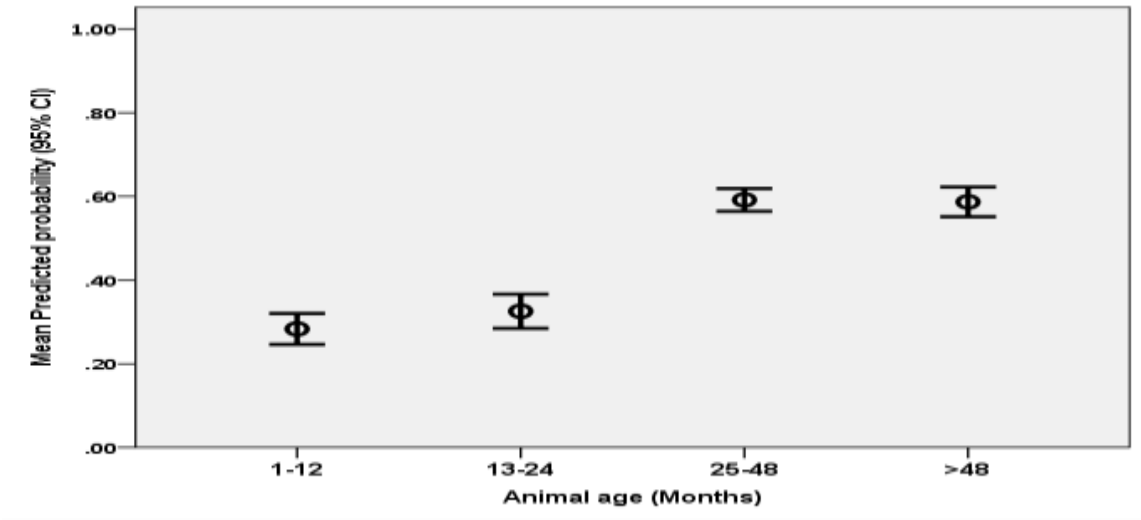

Figure 4-5: Mean predicted probabilities for CCHFV seropositivity by cattle age group.

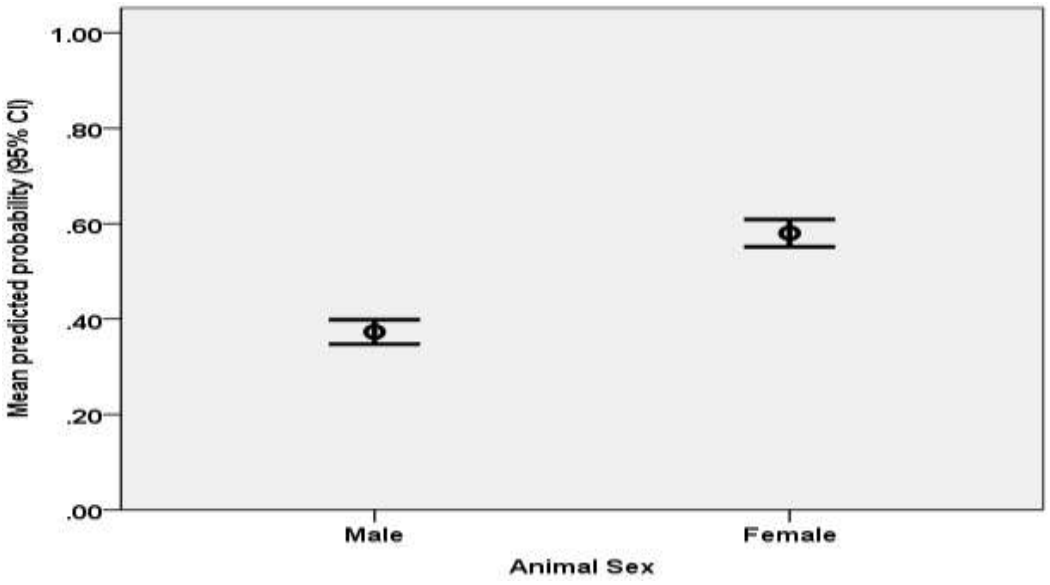

Figure 4-6: Mean predicted CCHFV seropositivity (95% CI) by cattle sex

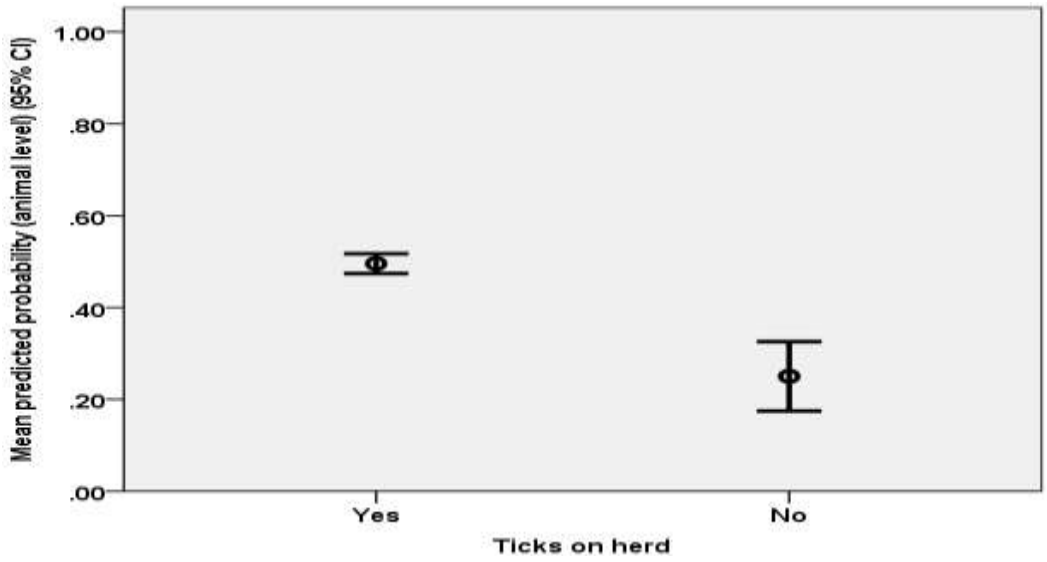

Figure 4-7: Mean predicted probabilities of CCHFV seropositivity by the availability of ticks on cattle.

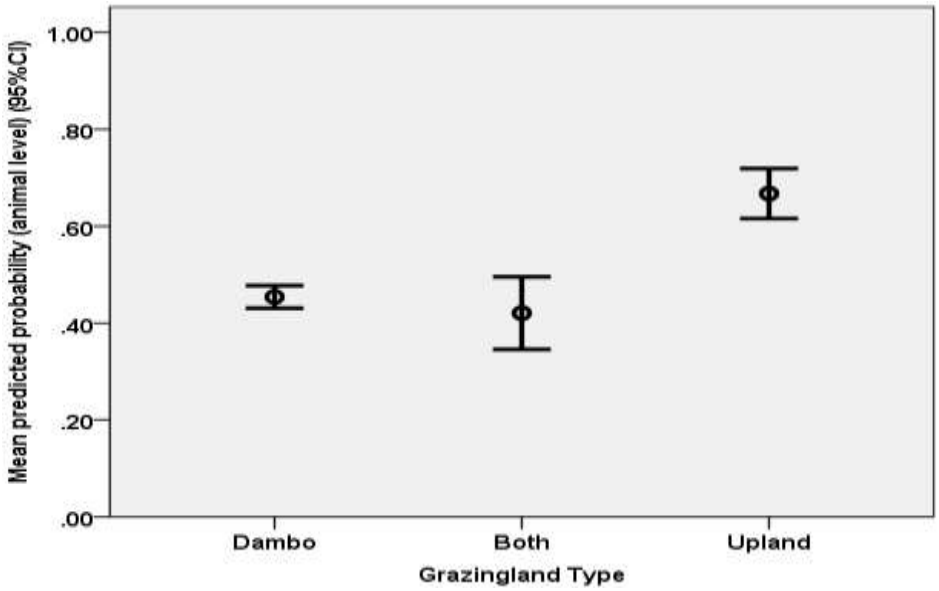

Figure 4-8: Mean predicted probability of CCHFV seropositivity by the type of grazing land

4.3 SEROPREVALENCE OF CCHV AT HERD LEVEL

Cattle from 117 herds were sampled and 109 farmers were interviewed. Eight farmers did not complete the questionnaire for various reasons not presented here. The overall herd-level prevalence of CCHF was 70.1% (82/117, 95% CI: 60.93-78.20) (Table 4-6). The

seroprevalence in Lilongwe ADD was 64.20 % (43/67, 95% CI: 51.53 - 75.53%) and 78.00% (39/50, 95% CI: 64.04 – 88.47%) in Kasungu ADD. Seropositivity for CCHFV significantly associated with district (p-value < 0.001), presence of ticks in the herd (p-value = 0.016) and cattle herd size (p-value = 0.224) (Table 4-6).

Table 4-1: Distribution of CCHFV seropositivity and risk factor association test with of CCHFV seropositivity at herd level

Risk Factor	Category	n	Seroprevalence (%)	95% CI	p-value
District	Dedza	18	83.3	58.58-96.42	<0.001*
	Dowa	13	76.92	46.19-94.96	
	Kasungu	13	61.54	31.58-86.14	
	Lilongwe East	34	38.24	22.17-56.44	
	Lilongwe West	15	100	78.20-100.0	
	Mchinji	17	82.4	56.57-96.20	
	Ntchisi	7	100	59.04-100.0	
Presence of ticks in the herd	Present	106	73.58	64.13-81.68	0.016*
	Absent	11	36.4	10.93-69.21	
Tick control	Present	67	73.1	60.9-83.24	0.287

	Absent	41	63.1	46.94-77.88	
Grazing land type	Dambo	94	70.2	59.9-79.21	0.469
	Both (Dambo and Upland)	12	58.3	27.67-84.83	
	Upland	11	81.8	39.03-93.98	
Herd size	Small	51	62.7	48.08-75.87	0.224*
	Medium	35	71.4	53.7-85.36	
	Large	31	80.6	62.53-92.55	

*Statistically significant at p<0.25; n= number of subjects; CI= confidence interval.

Table 4-7 shows the results for bivariate association tests between cattle farmer's level of education and farmer's perception towards tick significance on cattle with CCHFV seropositivity. CCHFV seropositivity did not significantly associate with neither farmer's level of education (p-value = 0.304) nor perception of farmers for tick significance towards cattle health (p-value = 0.729).

Table 4-2: Association test for herd owner related risk factors with cattle herd CCHF seroprevalence

Risk factor	Category	n	Seroprevalence (%)	95% CI	p-value
Highest level of education	None	8	75.0	34.91-96.81	0.304
	Primary	67	71.6	59.31-81.99	
	Secondary	29	58.6	38.94-76.48	
	Tertiary	4	100	39.76-100.0	
Tick significance (farmer perception)	Not important	10	80.0	55.5-99.75	0.729
	Important	30	70.0	50.6-85.27	
	Very Important	68	67.6	55.21-78.49	

n = number of cattle farmers; CI= Confidence interval; significance level ≤0.25

Table 4-8 summarizes the maximum likelihood estimates of the model built by significant factors in the bivariate analysis. The herd-level model built and used in this study fitted the observed data (Hosmer-Lemeshow test, p-value = 0.999), and its fixed components/variables significantly improved the model from the null model (Omnibus test of model coefficients, p-value = 0.001). The coefficients for district and tick presence were significant in the model (Table 4-8). The herds without ticks were less likely to be CCHFV seropositive (OR: 0.109; 95% CI: 0.017-0.71; p-value = 0.02) compared to those with ticks.

Table 4-3: Maximum likelihood estimates of herds being seropositive to CCHFV specific antibodies at herd level.

Risk factor	Category	OR	95% CI	P-value
District	Mchinji	r		
	Dedza	1.506	0.229-9.903	0.670
	Dowa	1.049	0.148-7.413	0.962
	Kasungu	0.300	0.055-1.633	0.164
	Lilongwe East	0.187	00.044-0.796	0.023***
Ticks on herd	Present	r		
	Absent	0.109	0.017-0.711	0.020***

*** Statistically significant at p-value <0.05; r=reference category, OR=Odds ratio, 95% CI= Confidence interval.

Figures 4-9 and 4-10 below show the plots of mean predicted probabilities for a herd being seropositive for CCHF against the independent variables that were significant in the model.

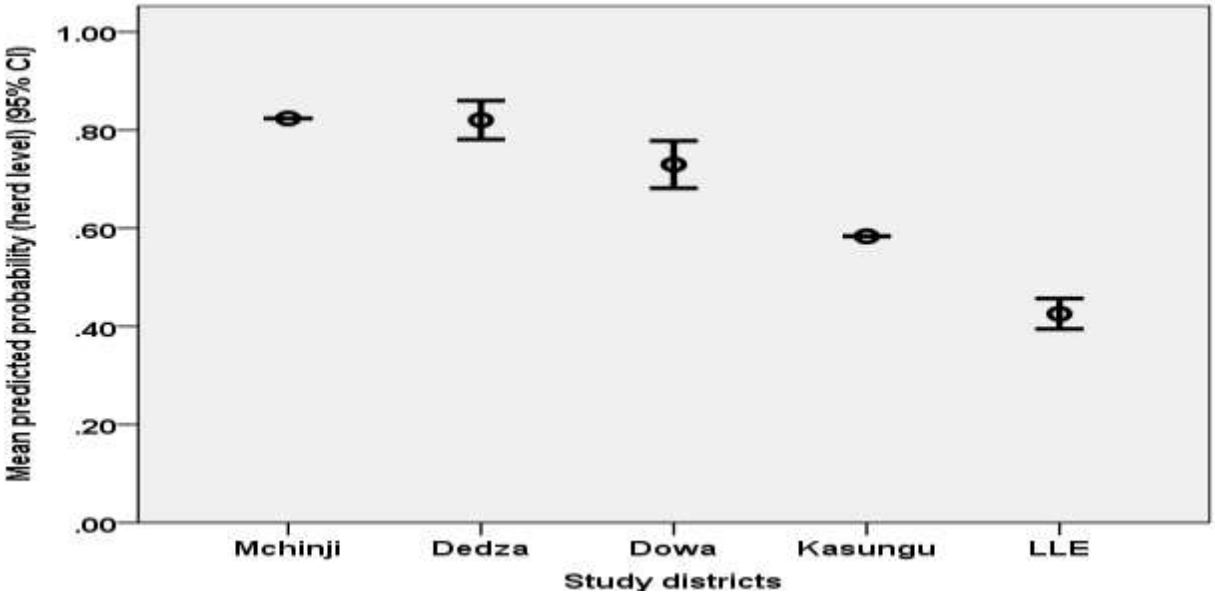

Figure 4-9: Mean predicted CCHFV seropositivity probabilities for a cattle herd in a particular district.

NB: LLE= Lilongwe East.

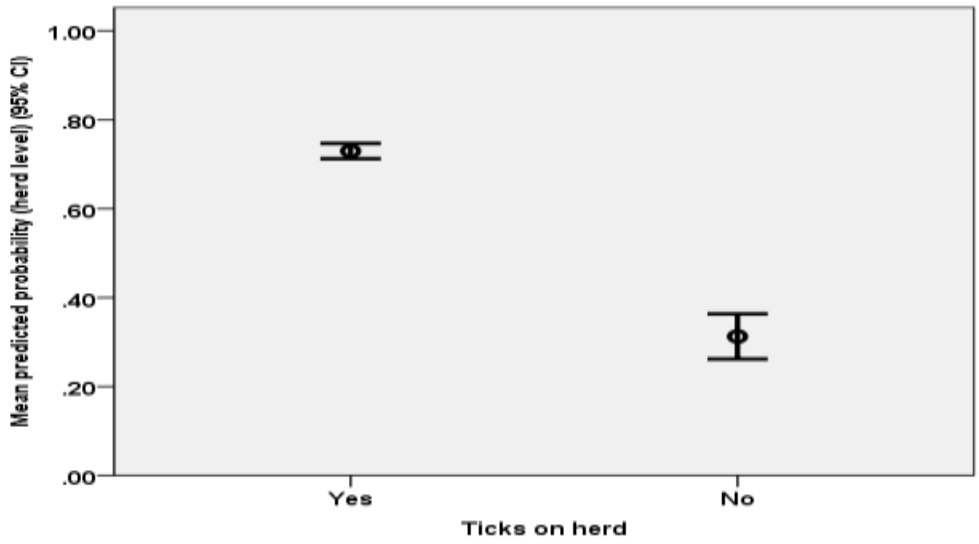

Figure 4-10: Mean predicted CCHFV seropositivity probabilities for a cattle herd with ticks

4.4 RT-PCR RESULTS

CCHFV S-segment genome was detected in 64.44% (29/45) pools of RNA from 296 individual ticks. Figure 11 shows observed amplified CCHFV S-segment region bands on gel electrophoresis.

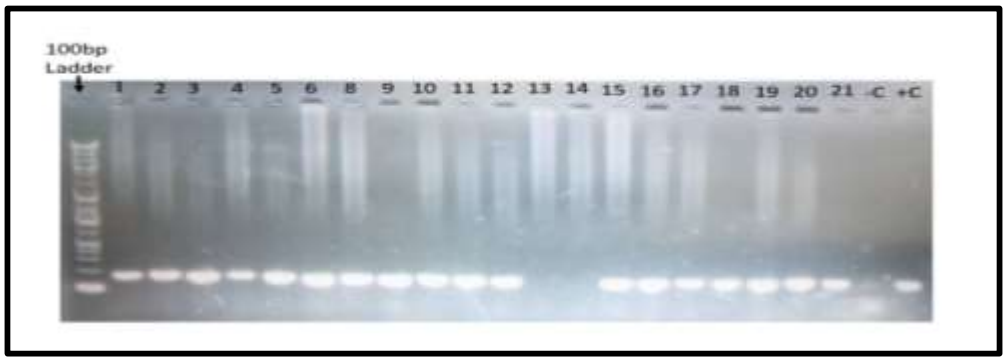

Figure 4-11: CCHFV S-segment amplified from RNA extracted from ticks

NB: 1-21= test samples; -C = negative control; and +C = positive control

The CCHFV S-segment detection rate per tick species was as follows: 9 out of 11 pools of H. truncatum, 18 out of 20 pools of A. variegetum, and 1 out of 12 pools of Rh. (B). decoloratus and none for Rh. (B). microplus and Rh. Appendiculatus.

CHAPTER FIVE

5.0 DISCUSSION

CCHFV has been reported in some African countries in humans, animals, or ticks. However, there are other countries whose CCHFV status is not yet known (Spengler et al., 2016; Tipih and Burt, 2020). Malawi was described as a high potential risk country for CCHF based on the presence of the principal vector (Hyalomma ticks) and having a conducive tropical climate (Pigott et al., 2017; Messina et al., 2015). For the first time, this study reports a high seroprevalence of 46.86% (195/416) of CCHFV in cattle coupled with the presence of CCHFV in ticks of different species in the central part of Malawi. These results provide further evidence of the CCHFV being endemic in Africa, thus even in those countries where no human cases of CCHF has previously been reported and are considered to be CCHF free.

CCHFV Seroprevalence in domesticated and wild animals varies among countries worldwide (Nasirian, 2019; Spengler et al., 2016). From a meta-analysis of data available by 2019, the global mean of CCHFV seroprevalence in cattle was estimated to be 18.6% (Nasirian, 2019). Higher seroprevalence of CCHFV in cattle, however, has been reported in some countries: 88.3% in Albania (Kadriaj et al., 2018), 75% in Uganda (Balinandi et al., 2021), and 69% in Mauritania (Schulz et al., 2021). Other CCHFV seroprevalence reported in sub-Saharan African countries includes Mali (66%), Senegal (57.1%) and DRC (0.4%), Sudan (19.4%) (Ibrahim et al., 2015) (Maiga et al., 2017; Sas et al., 2017; Mangombi et al., 2020; Spengler et al, 2016; Sadeuh-Mba et al., 2018; Balinandi et al., 2018). It can now be assumed that CCHFV is circulating in many countries in this sub-Saharan region undetected due to a lack of active surveillance, as was the case of Malawi. CCHFV seroprevalence being reported in this study (46.86%) is within the range of CCHFV seroprevalence being reported in cattle in other countries. It is worth noting that seroprevalence variations being reported by different studies are subject to CCHFV specific antibody detection methods used (Spengler, et al., 2016; Balinandi et al., 2021) other than true difference in prevalence. The multispecies CCHFV double antigen sandwich ELISA test used in this study detected both IgG and IgM (Sas et al., 2018) such that the CCHFV seropositivity being reported here is the sum of IgG and IgM seropositivity.

A number of risk factors are reported to be associated with CCHFV seropositivity. Location of animals had been reported to be significantly associated with seroprevalence of CCHFV as places vary in human activities (i.e. Livestock trade and farming); deforestation, presence of hosts and vectors; and climatic suitability (Balinandi et al., 2021; Sorvillo et al., 2020). The presence of forests and drier lands (in this case uplands) favor ground animals onto which immature Hyalomma ticks feed (Walker et al., 2014; Hoogstraal and Read, 2019), thus being more likely to have CCHFV circulating. Deforestation and settlement fragmentation that are common in the central of Malawi (Munthali and Murayama, 2013), disturb the ecology of small ground animals and forcing the vector ticks to infest livestock like cattle and henceforth transmit the virus (Sorvillo et al., 2020).

As cattle age, the cumulative period of exposure to ticks increases and so is the probability of being exposed to tick-borne diseases (Simuunza et al., 2011) such as CCHFV. Further, IgG has a long half-life hence chances of being detected even from longer past exposures are higher making older animals more likely to test positive. Similar observations were made in other studies (Ibrahim et al., 2015; Mangombi et al., 2020; Suliman, 2017; and Adam et al., 2013). The odds of cattle being seropositive for CCHF have been observed to be higher in female cattle than in males (Mangombi et al., 2020). Several explanations have been made for the association of female cattle and CCHFV seropositivity including: spending longer times on farms, thus the longer time of exposure to tick bites, as they are kept for breeding unlike male animals (Balinandi et al., 2021); the possibility of sexually transmitted infection as it has been reported in humans (Pshenichnaya et al., 2016), such that one male animal can transmit the virus to multiple females during breeding (Balinandi et al., 2021). In addition, for extensive communal grazing cattle, females spend most of their time in the fields grazing hence exposed to ticks compared to male cattle that are away from the grazing fields performing draught power. In contrast, Suliman (2017) and Adam et al (2013) reported no association between animal sex and CCHFV seropositivity.

The presence of other livestock species was not significantly associated with CCHF seropositivity in this study whilst Suliman (2017) and Adam et al (2013) observed a significant association between the two. In the current study, a larger proportion (98.2%) of farmers also kept other livestock species. Thus, there was no enough representation of farmers keeping

cattle only for a valid statistical comparison.

The Source of cattle, as being from the same district or another district, was significantly associated with CCHFV seropositivity in this study. Cattle from within the districts had a higher prevalence compared to those from outside a particular district. As the location is associated with CCHFV seropositivity, endemism of a particular location can influence the rate of transmission over time, locally.

The absence of ticks on cattle increased the likelihood of cattle being seropositive for CCHFV. Since ticks depend on blood meals from animals for survival, transmission from infected ticks to host animals would be more likely. Despite this being the case, some studies had reported no association between tick control and CCHFV seropositivity in cattle (Adam et al., 2013; Mangombi et al., 2020) but Suliman (2017) observed such an association in camels (Camelus dromedaries). However, treatment against ticks was not significantly associated with CCHFV seropositivity in this study, a finding that could be as a result of sampling following recent treatment against ticks that was followed by tick infestation. Further, cattle raised communally mingle during grazing with other cattle herds and other livestock in general, hence adherence to tick treatment protocols is required to ensure efficient prevention of transmission.

H. truncatum was found in all the districts except Dedza and Lilongwe West. These two districts also happened to be the top three districts with higher CCHFV seroprevalence: Dedza (57.1%; 95% CI: 43.22-70.29) and Lilongwe west (60.4%; 95% CI: 45.27-74.23). Possibly, other tick species including the H. rufipes, are transmitting the virus in the region. H. rufipes was reported in this region (Berggren, 1978) but could have been missed during sample collection because adults of this species are common at the beginning of the rain season (Walker et al., 2014) whilst sample collection, in this study, was done at the end of the rain season.

Cattle farmer's level of education and perceptions of tick significance on cattle health were not significantly associated with cattle being seropositive for CCHFV. Education and awareness of the importance of ticks were expected to promote good animal husbandry

practices which in turn would lower the odds of cattle being exposed to ticks and subsequently reduce CCHFV seropositivity. Further, with 62.04% (67/108) farmers treating their cattle herds against ticks and tick infestation level estimated at 90.59% (106/117) shows the inefficiency of the tick control strategies being applied in the study area. These results highlight, to some extent, inadequate knowledge in farmers to make informed decisions about good animal health management.

CCHFV S-genome detected in the ticks confirms circulation of the CCHFV among the ticks and assumes that cattle are exposed to the virus through tick bites locally. The CCHFV screening was done in pools of RNA due to limited reagents available for individual tick sample screening. Following these findings, sequencing of the detected CCHFV S-genome would provide epidemiological information on the CCHFV clade and strain circulating in central Malawi. The information from phylogenetic analysis would help to understand the geographical relationship of the CCHFV circulating in Malawi and other published CCHFV strains globally.

The exposure of cattle to CCHFV and detection of CCHFV in ticks strongly suggest that humans could also be exposed to this virus (Abbas et al., 2017; Mourya et al., 2012) in the central part of Malawi. Since CCHFV is a tick-borne zoonotic virus, causing a highly fatal disease, these findings are invaluable and informative to the public health sector in Malawi and call for urgent action from public health authorities.

The study was limited in coverage since it was done in the central part of Malawi. Hence the results cannot be extrapolated to the other regions of the country with different geographical and climatic patterns. Other risk factors such as season could not be studied because of the nature of the study (cross-sectional).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSIONS

Antibodies specific for CCHFV were found in cattle (46.9% seropositivity) and CCHFV S-segment was detected in ticks feeding on the cattle thus strongly suggesting possible exposure of cattle and circulation of CCHFV in the central region of Malawi. Animal age, animal sex, presence of ticks, locality and grazing areas were found to be significant risk factors of CCHF seropositivity in this cattle population.

6.2 RECOMMENDATIONS

The government, health surveillance policymakers, and private partner stakeholders should now consider CCHF in Malawi as a potential health risk requiring routine surveillance.

Raising community awareness on the risk of CCHF among the cattle farmers and other communities at risk.

Sequencing of the CCHFV genome is for molecular epidemiological analysis.

A country-wide survey is required both in humans and animals to explore the hotspots of CCHFV and CCHF in the country.

CHAPTER SEVEN

7.0 REFERENCES

Abbas, T., Z, Xu, M.Younus, A. Qayyum, and M. T. Riaz., (2017). 'Seasonality in hospital admissions of Crimean-Congo hemorrhagic fever and its dependence on ambient temperature—empirical evidence from Pakistan', International Journal of Biometeorology, 61(11), pp. 1893–1897. doi: 10.1007/s00484-017-1359-4.

Adam, I. A., M. A. M.Mahmoud, and I. E.Aradaib, (2013), 'A seroepidemiological survey of Crimean Congo hemorrhagic fever among Cattle in North Kordufan State, Sudan', Virology Journal, 10(1), p. 1. doi: 10.1186/1743-422X-10-178.

Akıncı, E., H. Bodur, and H. Leblebicioglu, (2015). 'Pathogenesis of Crimean-Congo Hemorrhagic Fever', (May 2013). doi: 10.1089/vbz.2012.1061.

Akuffo, R., J.A.M, Brandful, A. Zayed, A. Adjei, N. Watany, N. T. Fahmy, R. Hughes, B. Doman, S. V. Voegborlo, D. Aziati, D. Pratt, J. A. Awuni, N. Adams and E. Dueger., (2016). 'Crimean-Congo hemorrhagic fever virus in livestock ticks and animal handler seroprevalence at an abattoir in Ghana', BMC Infectious Diseases, 16(1), pp. 1–5. doi: 10.1186/s12879-016-1660-6.

Al-Abri, S. S. I. Abaidanib, M. Fazlalipourc, E. Mostafavid, H. Leblebicioglue, N. Pshenichnayaf, Z. A. Memishg, R. Hewsonh, E. Peterseni, P. Malaj, T M N. Nguyenj, M. R. Malikj, P. Formentyk, R. Jeffries, (2017). 'Current status of Crimean-Congo haemorrhagic fever in the World Health Organization Eastern Mediterranean Region: issues, challenges, and future directions', International Journal of Infectious Diseases, 58, pp. 82–89. doi: 10.1016/j.ijid.2017.02.018.

Anagnostou, V. and A. Papa, (2009). 'Evolution of Crimean-Congo Hemorrhagic Fever virus', Infection, Genetics and Evolution, 9(5), pp. 948–954. doi: 10.1016/j.meegid.2009.06.018.

Appannanavar, S. B. and B.Mishra, (2011). 'An update on crimean congo hemorrhagic fever', Journal of Global Infectious Diseases, 3(3), pp. 285–292. doi: 10.4103/0974-777X.83537.

Aradaib, I. E., B. R. Erickson, M.S. Karsany, M. L. Khristova, R. M. Elageb, M. E. H. Mohamed, S. T. Nichol, (2011). 'Multiple Crimean-Congo hemorrhagic fever virus strains are associated with disease outbreaks in Sudan, 2008-2009', PLoS Neglected Tropical Diseases, 5(5), pp. 2008–2009. doi: 10.1371/journal.pntd.0001159.

Arasli, M., (2016). ‘Pathogenesis of Crimean–Congo Hemorrhagic Fever From an Immunological Perspective’, Current Tropical Medicine Reports, 3(1), pp. 14–19. doi: 10.1007/s40475-016-0068-0.

Arteaga, L. M., J. L. M. Bellido, M. C. V. Lista, M. B. V. Santiago, P.F Soto, I. Bas, N. Leralta, F. de Ory Manchón, A. I. Negredo, M. Paz Sánchez Seco, M. A. Sardón, S. P. González, A. J. del Bianco, L. B. Peris, R. Alamo-Sanz, R. Hewson, M. Belhassen-García, A. Muro, (2020). ‘Crimean-Congo haemorrhagic fever (CCHF) virusspecific antibody detection in blood donors, Castile-León, Spain, summer 2017 and 2018’, Eurosurveillance, 25(10), pp. 1–7. doi: 10.2807/1560-7917.ES.2020.25.10.1900507.

Bakir, M., M. Ugurlu, B. Dokuzoguz, H. Bodur, M.A. Tasyaran, H. Vahaboglu and the Turkish CCHF Study Group, (2005). ‘Crimean-Congo haemorrhagic fever outbreak in Middle Anatolia: A multicentre study of clinical features and outcome measures’, Journal of Medical Microbiology, 54(4), pp. 385–389. doi: 10.1099/jmm.0.45865-0.

Balinandi, S., K. Patelb, J. Ojwangc, J. Kyondod, S. Muleid, A. Tumusiimea, B. Lubwamae, L. Nyakarahukad, J. D. Klenab, J. Lutwamad, U. Ströherb, S. T. Nicholb, and T. R. Shoemaker, (2018). ‘Investigation of an isolated case of human Crimean–Congo hemorrhagic fever in Central Uganda, 2015’, Int J Infect Dis., pp. 88–93. doi: 10.1016/j.ijid.2018.01.013.Investigation.

Balinandi, S., C. Br¨omssen, A. Tumusiime, J. Kyondo, H. Kwon, V. M. Monteil, A. Mirazimi, J. Lutwama, L. Mugisha and M. Malmberg, (2021). ‘Serological and molecular study of Crimean-Congo hemorrhagic fever virus in cattle from selected districts in Uganda’, Journal of Virological Methods, 290(December 2020), p. 114075. doi: 10.1016/j.jviromet.2021.114075.

Barker, S. C., A. R.Walker, and D.Campelo, (2014). ‘A list of the 70 species of Australian ticks; diagnostic guides to and species accounts of Ixodes holocyclus (paralysis tick), Ixodes cornuatus (southern paralysis tick) and Rhipicephalus australis (Australian cattle tick); and consideration of the place ’, International Journal for Parasitology, 44(12), pp. 941–953. doi: 10.1016/j.ijpara.2014.08.008.

Bartolini, B., C.E.M. Gruber, M. Koopmans, T. Avšič, S. Bino, I. Christova, R. Grunow, R. Hewson, G. Korukluoglu, C.M. Lemos, A. Mirazimi, A. Papa, M.P. Sanchez-Seco, A.V. Sauer, H. Zeller, C. Nisii, M. R. Capobianchi, G. Ippolito, C.B. Reusken, A. Di Caro, (2019).

‘Laboratory management of Crimean-Congo haemorrhagic fever virus infections: perspectives from two European networks.’, Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin, 24(5). doi: 10.2807/1560-7917.ES.2019.24.5.1800093.

Bente .D.A, N.L.Forester, D.M. Watts, A.J. McAuley, C.A.Whitehouse, M. Bray, (2013). ‘Crimean-Congo hemorrhagic fever: History, epidemiology, pathogenesis, clinical syndrome and genetic diversity’, Antiviral Research, 100(1), pp. 159–189. Available at: http://0-ovidsp.ovid.com.wam.city.ac.uk/ovidweb.cgi?T=JS&PAGE=reference&D=emed11&NEWS=N&AN=2013741328.

Bente .D.A, N.L.Forester, D.M. Watts, A.J. McAuley, C.A.Whitehouse, M. Bray, (2013). ‘Crimean-Congo hemorrhagic fever: History, epidemiology, pathogenesis, clinical syndrome and genetic diversity’, Antiviral Research, 100(1), pp. 159–189. doi: 10.1016/j.antiviral.2013.07.006.

Bergeron, É., M. Zivcec, A. K. Chakrabarti, S. T. Nichol, C. G. Albariño, C. F. Spiropoulou, (2015). ‘Recovery of Recombinant Crimean Congo Hemorrhagic Fever Virus Reveals a Function for Non-structural Glycoproteins Cleavage by Furin’, PLoS Pathogens, 11(5), pp. 1–19. doi: 10.1371/journal.ppat.1004879.

Berggren, S. A., (1978). ‘CATTLE TICKS IN MALAWI’, Veterinary Parasitology, 4, pp. 289–297.

Burt, F. J., R.Swanepoel, and L. E. O.Braack, (1993).‘Enzyme-linked immunosorbent assays for the detection of antibody to Crimean-Congo haemorrhagic fever virus in the sera of livestock and wild vertebrates’, Epidemiology and Infection, 111(3), pp. 547–558. doi: 10.1017/S0950268800057277.

Burt F.J. and R.Swanepoel, (2005). ‘Molecular epidemiology of African and Asian Crimean-Congo haemorrhagic fever isolates’, Epidemiology and Infection, 133, pp. 659–666.

Cajimat, M. N. B., S. E. Rodriguez, I. U.E. Schuster, D. M. Swetnam, T. G. Ksiazek, M. A. Habela, A. I. Negredo, A. Estrada-Pen˜a, A. D.T. Barrett and D. A. Bente, (2017). ‘Genomic Characterization of Crimean–Congo Hemorrhagic Fever Virus in Hyalomma Tick from Spain, 2014 ’, Vector-Borne and Zoonotic Diseases, 17(10), pp. 714–719. doi: 10.1089/vbz.2017.2190.

Center for Disease Control and Prevention (2019) ‘Congo Fever — Crimean-Congo

Hemorrhagic Fever', CRC Handbook of Viral and Rickettsial Hemorrhagic Fevers, pp. 121–130. doi: 10.1201/9780429276736-9.

Chauhan, R. P., Z. G. Dessie, A. Noreddin and M. E. El Zowalaty, (2020). 'Systematic review of important viral diseases in africa in light of the "one health" concept', Pathogens, 9(4). doi: 10.3390/pathogens9040301.

Chen, S., (2013). 'Molecular evolution of Crimean-Congo hemorrhagic fever virus based on complete genomes', Journal of General Virology, 94(PART4), pp. 843–850. doi: 10.1099/vir.0.049379-0.

Chinikar, S., N. Shah-Hosseini, S. Bouzari, M. Shokrgozar, E. Mostafavi, T. Jalali, S. Khakifirouz, M. H Groschup, M. Niedrig, (2016). 'Assessment of Recombination in the S-segment Genome of Crimean- Congo Hemorrhagic Fever Virus in Iran', J Arthropod-Borne Dis, 10(March), pp. 12–23.

Conger, N. G. K. M. Paolino, E. C. Osborn, J. M. Rusnak, S. Günther, J. Pool, P. E. Rollin, P. F. Allan, J. Schmidt-Chanasit, T. Rieger, and M. G. Kortepeter, (2015). 'Health care response to CCHF in US soldier and nosocomial transmission to health care providers, Germany, 2009', Emerging Infectious Diseases, 21(1), pp. 23–31. doi: 10.3201/eid2101.141413.

Coskun, U. S. S. and Z Asik,. (2019) 'Genotypic analysis of S segment of Crimean-Congo hemorrhagic fever virus in Turkey genotypic analysis of s segment of crimean-congo hemorrhagic fever virus in Turkey', Acta Microbiologica et Immunologica Hungarica, 66(September 2018), pp. 79–89. doi: 10.1556/030.65.2018.041.

Davies, F. G., (1997). 'Nairobi sheep disease', Parassitologia, 39(2), pp. 95–98.

Department of Animal Health And Livestock Development (DAHLD), (2006). 'DEVELOPMENT POLICY DOCUMENT ON LIVESTOCK IN MALAWI', (01). http://extwprlegs1.fao.org/docs/pdf/mlw169552.pdf

Deyde, V. M., M. L. Khristova, P. E. Rollin, T. G. Ksiazek, and S. T. Nichol, (2006). 'Crimean-Congo Hemorrhagic Fever Virus Genomics and Global Diversity', Journal of Virology, 80(17), pp. 8834–8842. doi: 10.1128/jvi.00752-06.

Dohoo. l., W. Martin, and H. Stryhn, (2004). 'Veterinary Epidemiologic Research, Applied Animal Behaviour Science'. doi: 10.1016/j.applanim.2004.03.001.

Dowall, S. D., K. R Buttigieg, S. J. D. Findlay-Wilson, E. Rayner, G. Pearson, A.

Miloszewska, V.A. Graham, M.W. Carroll, and R. Hewson, (2015). 'Human Vaccines & Immunotherapeutics A Crimean-Congo Haemorrhagic Fever (CCHF) viral vaccine expressing nucleoprotein is immunogenic but fails to confer protection against lethal disease A Crimean-Congo Haemorrhagic Fever (CCHF) viral vaccine expressin', (September). doi: 10.1080/21645515.2015.1078045.

Drosten, C., B. M. Kümmerer, H. Schmitz, S. Günther, (2003). 'Molecular diagnostics of viral hemorrhagic fevers', Antiviral Research, 57(1–2), pp. 61–87. doi: 10.1016/S0166-3542(02)00201-2.

Duh, D., S. T. Nichol, M. L Khristova, A Saksida, I. Hafner-Bratkovič, M. Petrovec, I. Dedushaj, S. Ahmeti and T. Avšič-Županc, (2008). 'The complete genome sequence of a Crimean-Congo Hemorrhagic Fever virus isolated from an endemic region in Kosovo', Virology Journal, 6, pp. 1–6. doi: 10.1186/1743-422X-5-7.

Ergonul, O., (2012). 'Crimean-Congo hemorrhagic fever virus: New outbreaks, new discoveries', Current Opinion in Virology, 2(2), pp. 215–220. doi: 10.1016/j.coviro.2012.03.001.

Escadafal, C., S. O¨ lschla¨ger, T. Avsˇicˇ-Zˇ upanc, A. Papa, J. Vanhomwegen, R. Wo¨ lfel, A Mirazimi, A. Teichmann, O. Donoso-Mantke and M. Niedrig, (2012). 'First international external quality assessment of molecular detection of crimean-congo hemorrhagic fever virus', PLoS Neglected Tropical Diseases, 6(6). doi: 10.1371/journal.pntd.0001706.

Esmon, C. T., (2004). 'Interactions between the innate immune and blood coagulation systems', Trends in Immunology, 25(10), pp. 536–542. doi: 10.1016/j.it.2004.08.003.

Estrada, D. F. and R. N. De Guzman, (2011). 'Structural characterization of the Crimean-Congo hemorrhagic fever virus Gn tail provides insight into virus assembly', Journal of Biological Chemistry, 286(24), pp. 21678–21686. doi: 10.1074/jbc.M110.216515.

Fajs, L., Jakupi, X. Ahmeti, I. Humolli, I. Dedushaj, and T. Avsˇicˇ-Zˇ upanc, (2014). 'Molecular Epidemiology of Crimean-Congo Hemorrhagic Fever Virus in Kosovo', PLoS Neglected Tropical Diseases, 8(1), p. 43. doi: 10.1371/journal.pntd.0002647.

Fajs, L., I. Humolli, A. Saksida, N. Knap, M. Jelovsˇek, M. Korva, I. Dedushaj, T. Avsˇicˇ-Zˇ upanc, (2014). 'Prevalence of Crimean-Congo hemorrhagic fever virus in healthy population, livestock and ticks in Kosovo', PLoS ONE, 9(11), pp. 8–10. doi: 10.1371/journal.pone.0110982.

Food and Agriculture organisation (FAO), (2009) 'Country Report on the State of Plant Genetic Resources for Food and Agriculture: Malawi'.

Gale, P., B. Stephenson, A. Brouwer, M. Martinez, A. de la Torre, J. Bosch, M. Foley-Fisher, P. Bonilauri, A. Lindstro¨m, R.G. Ulrich, C.J. de Vos, M. Scremin, Z. Liu, L. Kelly and M.J. Mun˜ oz, (2012). 'Impact of climate change on risk of incursion of Crimean-Congo haemorrhagic fever virus in livestock in Europe through migratory birds', Journal of Applied Microbiology, 112(2), pp. 246–257. doi: 10.1111/j.1365-2672.2011.05203.x.

Gargili, A., A. Estrada-Peñad, J. R. Spenglere, A. Lukashevf, P.A. Nuttallh, and D. A. Bente, (2018). 'HHS Public Access', pp. 93–119. doi: 10.1016/j.antiviral.2017.05.010.

Garrison, A. R., S. R. Radoshitzky, K. P. Kota, G. Pegoraro, G. Ruthel, J. H. Kuhn, L. A. Altamura, S. A. Kwilas, S. Bavari, V Haucke, C. S. Schmaljohn, (2013). 'Crimean-Congo hemorrhagic fever virus utilizes a clathrin- and early endosome-dependent entry pathway', Virology, 444(1–2), pp. 45–54. doi: 10.1016/j.virol.2013.05.030.

Garrison, A. R., D. R.Smith, and J. W.Golden, (2019). 'Animal Models for Crimean-Congo Hemorrhagic Fever Human Disease', Viruses, 11. doi: 10.3390/v11070590.

Gonzalez, J. P., M.L. Wilson, J.P. Cornet and J.L. Camicas, (1995). 'Host-passage-induced phenotypic changes in Crimean-Congo haemorrhagic fever virus', Research in Virology, 146(2), pp. 131–140. doi: 10.1016/0923-2516(96)81082-X.

Goswami, T K., D. K. Singh, M. Saminathan, A. K. Verma, and K. Dhama, (2014). 'An Emerging Threat of Crimean Congo Hemorrhagic Fever: Call for Preparedness', Advances in Animal and Veterinary Sciences, 2(1), pp. 8–14. doi: 10.14737/journal.aavs/2014.2.1.8.14.

Grandi, G., L. Chitimia-Doblerc, P. Choklikitumnueya, C. Strubee, A. Springere, A. Albihna, T. G. T. Jaensong, A. Omazic, (2020). 'First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden', Ticks and Tick-borne Diseases, 11(3), pp. 1–5. doi: 10.1016/j.ttbdis.2020.101403.

Hassanein, K. M., O. M. E.El-Azazy, and H. M.Yousef, (1997). 'Detection of Crimean-Congo haemorrhagic fever virus antibodies in humans and imported livestock in Saudi Arabia', Transactions of the Royal Society of Tropical Medicine and Hygiene, 91(5), pp. 536–537. doi: 10.1016/S0035-9203(97)90014-6.

Hawman, D. W. and H. Feldmann, (2018). 'Recent advances in understanding Crimean–Congo hemorrhagic fever virus', F1000Research, 7(0), p. 1715. doi:

10.12688/f1000research.16189.1.

Hewson, R., J. Chamberlain, V. Mioulet , G. Lloyd, B. Jamil , R. Hasan, A. Gmyl, L Gmyl, S.E. Smirnova, A. Lukashev, G. Karganova, C. Clegg, (2004). 'Crimean-Congo haemorrhagic fever virus: Sequence analysis of the small RNA segments from a collection of viruses world wide', Virus Research, 102(2), pp. 185–189. doi: 10.1016/j.virusres.2003.12.035.

Hewson, R., A. Gmyl, L. Gmyl, S. E. Smirnova, G. Karganova, B. Jamil, R. Hasan, J. Chamberlain and C. Clegg, (2004). 'Evidence of segment reassortment in Crimean-Congo haemorrhagic fever virus Printed in Great Britain', pp. 3059–3070. doi: 10.1099/vir.0.80121-0.

Hewson, R., (2007). 'Molecular epidemiology, genomics, and phylogeny of Crimean-Congo hemorrhagic fever virus', Crimean-Congo Hemorrhagic Fever: A Global Perspective, pp. 45–55. doi: 10.1007/978-1-4020-6106-6_5.

Hoogstraal, H., (1979). 'The epidemiology of tick-borne Crimean-Congo Hemorrhagic Fever in Asia, Europe, and Africa', journal of medical entomology, 15(4), pp. 307–417. doi: 10.1093/jmedent/15.4.307.

Hoogstraal, H., (1981). 'Changing Patterns of Tickborne Diseases in Modern Society', Annual Review of Entomology, 26(1), pp. 75–99. doi: 10.1146/annurev.en.26.010181.000451.

Hoogstraal, H. and M.Read, (2019). 'Review Article : The Epidemiology of Tick-Borne Crimean-Congo Hemorrhagic Fever in Asia , Europe , and Africa Latest Sign in Oxford Academic account Entomological Society of America members Sign in via your Institution Short-term Access', p. 2019.

Ibrahim, A. M., I. A. Adam, B. T. Osman, I. E. Aradaib, (2015). 'Epidemiological survey of Crimean Congo hemorrhagic fever virus in cattle in East Darfur State, Sudan', Ticks and Tick-borne Diseases, 6(4), pp. 439–444. doi: 10.1016/j.ttbdis.2015.03.002.

Ince, Y. ., C. Yasa, M. Metin, M. Sonmez, E. Meram, B. Benkli, O. Ergonul, (2014). 'International Journal of Infectious Diseases Crimean-Congo hemorrhagic fever infections reported by ProMED', International Journal of Infectious Diseases, 26, pp. 44–46. doi: 10.1016/j.ijid.2014.04.005.

Jauréguiberry, S., P. Tattevin, A. Tarantola, F. Legay, A. Tall, P. Nabeth, H. Zeller, and C. Michelet, (2005). 'Imported Crimean-Congo hemorrhagic fever', Journal of Clinical Microbiology, 43(9), pp. 4905–4907. doi: 10.1128/JCM.43.9.4905-4907.2005.

Jeeva, S., E. Cheng, S. S. Ganaie, M. A. Mir, (2017). 'Crimean-Congo Hemorrhagic Fever Virus Nucleocapsid Protein Augments mRNA Translation', Journal of Virology, 91(15), pp. 1–18. doi: 10.1128/jvi.00636-17.

Kadriaj, P., M. Dhimolea-Kota, E. Velo, K. Mersini, A. Simaku, K. Berxholi, S. Bino, (2018). 'Seroepidemiology of CCHF in domestic animals in endemic areas in Albania', Academic Journal of Interdisciplinary Studies, 7(2), pp. 25–29. doi: 10.2478/ajis-2018-0043.

Kaiser, M. N. and H. Hoogstraal, (1963). 'The Hyalomma ticks (Ixodoidea, Ixodidade) of Afghanistan.', The Journal of parasitology, 49(1), pp. 130–139. doi: 10.2307/3275691.

Leblebicioglu, H., (2010). 'Crimean-Congo haemorrhagic fever in Eurasia', International Journal of Antimicrobial Agents, 36(SUPPL. 1), pp. 43–46. doi: 10.1016/j.ijantimicag.2010.06.020.

Leblebicioglu, H., M. Sunbul, Z.A. Memish, J. A. Al-Tawfiq, H. Bodur, A. Ozkul, A. Gucukoglu, S. Chinikar, Z. Hasan, (2015). 'Consensus report: Preventive measures for Crimean-Congo Hemorrhagic Fever during Eid-al-Adha festival', International Journal of Infectious Diseases, 38, pp. 9–15. doi: 10.1016/j.ijid.2015.06.029.

Leblebicioglu, H., R. Ozaras, T. E. Fletcher, Nick J. Beeching, (2016). 'Crimean-Congo haemorrhagic fever in travellers: A systematic review', Travel Medicine and Infectious Disease, 14(2), pp. 73–80. doi: 10.1016/j.tmaid.2016.03.002.

Li, G., J. P. Messina, B. G. Peter, S, S. Snapp, (2017). 'Mapping Land Suitability for Agriculture in Malawi', Land Degradation and Development, 28(7), pp. 2001–2016. doi: 10.1002/ldr.2723.

Lukashev, A. N., (2005) 'Evidence for recombination in Crimean-Congo hemorrhagic fever virus', pp. 2333–2338. doi: 10.1099/vir.0.80974-0.

Maes, P., S. V. Alkhovsky, Y. Bào, M. Beer, M. Birkhead, T. Briese, M. J. Buchmeier, C. H. Calisher, R. N. Charrel, I. R. Choi, C. S. Clegg, J. C. de la Torre, E. Delwart, J. L. DeRisi, P. L. Di Bello, F. Di Serio, M. Digiaro, V. V. Dolja, C. Drosten, T. Z. Druciarek, J. Du, H. Ebihara, T. Elbeaino, R. C. Gergerich et al., (2019). 'Taxonomy of the order Bunyavirales: second update 2018', Archives of Virology, 164(3), pp. 927–941. doi: 10.1007/s00705-018-04127-3.

Maiga, O., M. A. Sas, K. Rosenke, B. Kamissoko, M. Mertens, N. Sogoba, A. Traore, M. Sangare, M. Niang, T. G. Schwan, H. M. Maiga, S. F. Traore, H. Feldmann, D. Safronetz and

M. H. Groschup, (2017). ‘Serosurvey of Crimean-Congo hemorrhagic fever virus in Cattle, Mali, West Africa’, American Journal of Tropical Medicine and Hygiene, 96(6), pp. 1341–1345. doi: 10.4269/ajtmh.16-0818.

Mangombi, J. B., C. Roqueplo, M. Sambou, M. Dahmani, O. Mediannikov, L. Comtet and B. Davoust, (2020). ‘Seroprevalence of Crimean-Congo Hemorrhagic Fever in Domesticated Animals in Northwestern Senegal’, Vector-Borne and Zoonotic Diseases, 20(10), pp. 797–799. doi: 10.1089/vbz.2019.2592.

Mares., R. G., (1964). ‘Animal Health and Production’, Australian Veterinary Journal, 40(9), pp. 339–339. doi: 10.1111/j.1751-0813.1964.tb04551.x.

Mathison, B. A., W. J. Gerth, B. S. Pritt and S. Baugh, (2015). ‘Introduction of the exotic tick Hyalomma truncatum on a human with travel to Ethiopia: A case report’, Ticks and Tick-borne Diseases, 6(2), pp. 152–154. doi: 10.1016/j.ttbdis.2014.11.005.

Mazzola, L. T. and C.. Kelly-Cirino, (2019). ‘Diagnostic tests for Crimean-Congo haemorrhagic fever: A widespread tickborne disease’, BMJ Global Health, 4. doi: 10.1136/bmjgh-2018-001114.

Mehanda, M., F. Al-Shorbajia, P. Millettb, B. Murgue, (2018). ‘The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development effort’, Antiviral Research 159 (2018) 63–67, (January). doi: org/10.1016/j.antiviral.2018.09.009.

Mertens, M., Z. Vatansever , S. Mrenoshki, K. Krstevski, J. Stefanovska, I. Djadjovski, et al, (2015). ‘Circulation of Crimean-Congo Hemorrhagic Fever Virus in the Former Yugoslav Republic of Macedonia Revealed by Screening of Cattle Sera Using a Novel Enzyme-linked Immunosorbent Assay’, PLoS Neglected Tropical Diseases, 9(3), pp. 1–10. doi: 10.1371/journal.pntd.0003519.

Messina, J. P., D. M. Pigott, N. Golding, K. A. Duda, J. S. Brownstein, D. J. Weiss, H. Gibsona, T. P. Robinson, M. Gilberte, G. R. W. Winta, P. A. Nuttalla, P. W. Gethinga, M. F. Myersa, D. B. Georgeg and S. I. Hay, (2015). ‘The global distribution of Crimean-Congo hemorrhagic fever’, Transactions of the Royal Society of Tropical Medicine and Hygiene, 109(8), pp. 503–513. doi: 10.1093/trstmh/trv050.

Moraga-Fernández, A., F, Ruiz-Fons, Miguel A. Habela, L. Royo-Hernández, R. Calero-Bernal, C. Gortazar, J. Fuente,I. G. F. de Mera1, (2020). ‘Detection of new Crimean–Congo

haemorrhagic fever virus genotypes in ticks feeding on deer and wild boar, Spain', Transboundary and Emerging Diseases, 1944(May), pp. 1–8. doi: 10.1111/tbed.13756.

Mostafavi, E., S. Chinikar, S, Bokaei,, A. Haghdoost, (2013). 'Temporal modeling of Crimean-Congo hemorrhagic fever in eastern Iran', International Journal of Infectious Diseases, 17(7), pp. e524–e528. doi: 10.1016/j.ijid.2013.01.010.

Mourya, D. T., P. D. Yadav., A. M. Shete, Y. K. Gurav, C. G. Raut, R. S. Jadi, S. D. Pawar, S. T. Nichol, Mishra, A. C., (2012). 'Detection, Isolation and Confirmation of Crimean-Congo Hemorrhagic Fever Virus in Human, Ticks and Animals in Ahmadabad, India, 2010–2011', PLoS Neglected Tropical Diseases. Edited by D. G. Bausch, 6(5), p. e1653. doi: 10.1371/journal.pntd.0001653.

Mourya D.T., P. D. Yadav, A. Shete, T. D. Majumdar, A. K. D. Kapadia, V. Chandra, A. J. Kachhiapatel, P. T. J. and K. J. Upadhyay, P. Dave, and D. R., (2014). 'Serosurvey of Crimean-Congo Hemorrhagic Fever Virus in Domestic Animals, Gujarat, India, 2013', VECTOR-BORNE AND ZOONOTIC DISEASES, 14(9), pp. 690–692. doi: 10.1089/vbz.2014.1586.

Muianga, A. F., R. Watson, A. Varghese, I. S. Chongoa, S. Ali, V. Monteiro, F. Inalda, I. Chelene, V. António, R. Hewson, E. S. Gudo, (2017). 'First serological evidence of Crimean-Congo haemorrhagic fever in febrile patients in Mozambique', International Journal of Infectious Diseases, 62, pp. 119–123. doi: 10.1016/j.ijid.2017.07.024.

Munthali, K. G. and Y. Murayama, (2013). 'Interdependences between smallholder farming and environmental management in rural Malawi: A case of agriculture-induced environmental degradation in Malingunde Extension Planning Area (EPA)', Land, 2(2), pp. 158–175. doi: 10.3390/land2020158.

Munyeme, M., J. B Muma, H. MMunang'andu, C. Kankya, E. Skjerve and M. Tryland, (2010). 'Cattle owners' awareness of bovine tuberculosis in high and low prevalence settings of the wildlife-livestock interface areas in Zambia', BMC Veterinary Research, 6. doi: 10.1186/1746-6148-6-21.

Munibullah, A. Yousaf, M.A. Shah, et al., (2018). 'Crimean – Congo Hemorrhagic Fever a Threat to Public Health .', Journal of Bacteriology and Infectious Diseases, 2(1), pp. 1–7.

Mushonga, B., I. Shinexuugi, P. Mbiri, A. Samkange, E. Kandiwa, (2020).'Applicability of teeth examination as a tool for age estimation in a semi-arid cattle production environment in

Namibia', Tropical Animal Health and Production, 52(4), pp. 1649–1654. doi: 10.1007/s11250-019-02172-x.

Nasirian, H., (2019). 'Crimean-Congo hemorrhagic fever (CCHF) seroprevalence : A systematic review and meta-analysis', Acta Tropica, 196(May), pp. 102–120. doi: 10.1016/j.actatropica.2019.05.019.

Nasirian, H., (2020). 'New aspects about Crimean-Congo hemorrhagic fever (CCHF) cases and associated fatality trends : A global systematic review and meta-analysis', Comparative Immunology, Microbiology and Infectious Diseases, 69(August 2019), p. 101429. doi: 10.1016/j.cimid.2020.101429.

National Statistics Office (N SO), (2018). National and District Population Projections 2018-2050.

OIE (2008). 'Collection and shipment of diagnostic specimens', in OIE Terrestrial Manual 2008, pp. 1–14. Available at: https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/1.1.01_COLLECTION.pdf.

Onguru, P., S. Dagdas, H. Bodur, M. Yilmaz, E. Akinci, S. Eren and G. Ozet, (2010). 'Coagulopathy parameters in patients with Crimean-Congo hemorrhagic fever and its relation with mortality', Journal of Clinical Laboratory Analysis, 24(3), pp. 163–166. doi: 10.1002/jcla.20383.

Ozdarendeli, A., K. Aydin, S. Tonbak, M. Aktas, K. Altay, I. Koksal, Y. Bolat, N. Dumanli, A. Kalkan, (2008). 'Genetic analysis of the M RNA segment of Crimean-Congo hemorrhagic fever virus strains in Turkey', Archives of Virology, 153(1), pp. 37–44. doi: 10.1007/s00705-007-1056-4.

Palomar, A. M., A. Portillo, P. Santibáñez, D. Mazuelas, J. Arizaga, A. Crespo, Ó. Gutiérrez, J. F. Cuadrado, and J. A. Oteo, (2013).'Crimean-congo hemorrhagic fever virus in ticks from migratory birds, Morocco', Emerging Infectious Diseases, 19(2), pp. 260–263. doi: 10.3201/eid1902.121193.

Palomar, A. M., A. Portillo, S. Santibá˜nez, L. García-Álvarezas, A. Mu˜noz-Sanz, F. J. Márquez, L. Romero, J. M. Eiros, and J. A. Oteo, (2017). 'Molecular (ticks) and serological (humans) study of Crimean-Congo hemorrhagic fever virus in the Iberian Peninsula, 2013–2015', Enfermedades Infecciosas y Microbiologia Clinica, 35(6), pp. 344–347. doi:

10.1016/j.eimc.2017.01.009.

Peyrefitte, C. N., M Perret, S. Garcia, R. Rodrigues, A. Bagnaud, S. Lacote, J. Crance, G. Vernet, D. Garin, M. le Bouloy and G. Paranhos-Baccala C. N. et al., (2010). 'Differential activation profiles of Crimean-Congo hemorrhagic fever virus- and Dugbe virus-infected antigen-presenting cells', Journal of General Virology, 91(1), pp. 189–198. doi: 10.1099/vir.0.015701-0.

Pica-Ciamarra, U., L. Tasciotti, L. Tasciotti , J. Otte and A. Zezza, (2011).'Livestock Assets , Livestock Income and Rural Households: Cross-Country Evidence from Household Surveys', World Bank, (May), p. 18. Available at: http://www.africalivestockdata.org/afrlivestock/sites/africalivestockdata.org/files/PAP_Livestock_HHSurveys.pdf.

Pigott, D. M., A. Deshpande, I. Letourneau, C. Morozoff, R. C. Reiner Jr, M. U. G .Kraemer, S. E.Brent, I. I. Bogoch, K. Khan, M. H. Biehl, R. Burstein, L. Earl, N. Fullman, J. P. Messina, A. Q. N. Mylne, C. L. Moyes, F. M. Shearer, S. Bhatt, O. J. Brady, P. W. Gething, D. J. Weiss, A. J. Tatem, L. Caley, T. De Groeve, L. Vernaccini, N. Golding, P. Horby, J. H. Kuhn, S. J. Laney, Edmond Ng, P. Piot, O. Sankoh, C. J. L. Murray, S. I. Hay, (2017).(2017) 'Articles Local , national , and regional viral haemorrhagic fever pandemic potential in Africa : a multistage analysis', The Lancet, 6736(17), pp. 1–11. doi: 10.1016/S0140-6736(17)32092-5.

Pshenichnaya, N. Y. et al. (2016). 'Possible sexual transmission of Crimean-Congo hemorrhagic fever', International Journal of Infectious Diseases, 45, pp. 109–111. doi: 10.1016/j.ijid.2016.02.1008.

Pshenichnaya, N. Y. and Nenadskaya, S. A., (2015). 'Probable Crimean-Congo hemorrhagic fever virus transmission occurred after aerosol-generating medical procedures in Russia: Nosocomial cluster', International Journal of Infectious Diseases, 33, pp. 120–122. doi: 10.1016/j.ijid.2014.12.047.

QIAGEN® OneStep RT-PCR Handbook, (2012). 'October 2012 For fast and highly sensitive one-step RT-PCR Sample & Assay Technologies QIAGEN Sample and Assay Technologies', (October).

Rehman, K., M. A. K. Bettani, L. Veletzky, S. Afridi and M. Ramharter. (2018),'Outbreak of Crimean-Congo haemorrhagic fever with atypical clinical presentation in the Karak District of Khyber Pakhtunkhwa, Pakistan', Infectious Diseases of Poverty, 7(1), pp. 1–6. doi:

10.1186/s40249-018-0499-z.

Richards, G. A., J.Weyer and L.Blumberg, (2015). 'Viral haemorrhagic fevers in South Africa', South African Medical Journal, 105(9), p. 748. doi: 10.7196/SAMJnew.8330.

Rickert-hartman, R. and J. P. Folster, (2014) 'Unique Strain of Crimean – Congo Hemorrhagic Fever', 20(5) 'Unique Strain of Crimean – Congo Hemorrhagic Fever', 20(5). Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA. doi: http://dx.doi.org/10.3201/eid2005.131575

Rodrigues, R., G. Paranhos-Baccala, G. Vernet, C. N. Peyrefitte, (2012).'Crimean-Congo hemorrhagic fever virus-infected hepatocytes induce ER-stress and apoptosis crosstalk', PLoS ONE, 7(1). doi: 10.1371/journal.pone.0029712.

Rodriguez, L. L., G. O. Maupin, T. G. Ksiazek, P. E. Rollin, A. S. Khan,T. E Schwarz, R. S. Lofts, J. F. Smith, A. M. Noor, C. J. Peters, And S. T. Nichol, (1997). 'Molecular investigation of a multisource outbreak of Crimean-Congo hemorrhagic fever in the United Arab Emirates', American Journal of Tropical Medicine and Hygiene, 57(5), pp. 512–518. doi: 10.4269/ajtmh.1997.57.512.

Sadeuh-Mba, S. A., G. M. Y. Wansi, M. Demanou, A. Gessain and R. Njouom, (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). doi: 10.1186/s12985-018-0977-8.

Sara Shayan, M. B., M. R.Shahrivar, and S. Chinikar, (2015). 'Crimean-Congo Hemorrhagic Fever', Lab Medicine, 46(3), pp. 180–189. doi: 10.1309/LMN1P2FRZ7BKZSCO.

Sas, M. A., M. Mertens, E. Isselmou, N. Reimer, B. O. El Mamy, B. Doumbia and M. H. Groschup, (2017). 'Crimean-Congo Hemorrhagic Fever Virus-Specific Antibody Detection in Cattle in Mauritania', Vector-Borne and Zoonotic Diseases, 17(8), pp. 582–587. doi: 10.1089/vbz.2016.2084.

Sas, M. A., L. Comtet, F. Donnet, M. Mertens, Z. Vatansever, N. Tordo, P. Pourquier, M. H. Groschup, (2018).'A novel double-antigen sandwich ELISA for the species-independent detection of Crimean-Congo hemorrhagic fever virus-specific antibodies', Antiviral Research. doi: 10.1016/j.antiviral.2018.01.006.

Schulz, A., Y. Barry, F. Stoek, M. J. Pickin, A. Ba, L. Chitimia, M. L. Haki, B. A. Doumbia, A. Eisenbarth, A. Diambar, M. Y. Bah, M. Eiden, M. H. Groschup, (2020).'Detection of

Crimean-Congo hemorrhagic fever virus (CCHFV) in Hyalomma ticks collected from Mauritanian livestock', pp. 1–20. doi: 10.21203/rs.3.rs-45301/v1.

Schulz, A., Y. Barry, F. Stoek, A. Ba, J. Schulz, M.L. Haki, et al., (2021).'Crimean-congo hemorrhagic fever virus antibody prevalence in mauritanian livestock (Cattle, goats, sheep and camels) is stratified by the animal's age', PLoS Neglected Tropical Diseases, 15(4), pp. 1–12. doi: 10.1371/journal.pntd.0009228.

Schuster, I., M. Mertens, S. Mrenoshki, C. Staubach, C. Mertens, F. Bru¨ning, K. Wernike, S. Hechinger, K. Berxholi, D. Mitrov, M. H. Groschup, (2016).'Sheep and goats as indicator animals for the circulation of CCHFV in the environment', Experimental and Applied Acarology, 68(3), pp. 337–346. doi: 10.1007/s10493-015-9996-y.

Shahhosseini, N., A. Jafarbekloo, Z. Telmadarraiy, S. Chinikar, A. Haeri, N. Nowotny, M. H. Groschup, A. R. Fooks, F. Faghihi N. et al., (2017). 'Co-circulation of Crimean-Congo Hemorrhagic Fever virus strains Asia 1 and 2 between the border of Iran and Pakistan', Heliyon, 3(11), p. e00439. doi: 10.1016/j.heliyon.2017.e00439.

Shayan, S., M. Bokaean, M. R. Shahrivar, S. Chinikar, (2019). 'Crimean-Congo Hemorrhagic Fever Crimean-Congo Hemorrhagic Fever Related articles in PubMed Phylogenetic Relationship Genetic Reassortment At-Risk Population Clinical Findings and Pathogenesis', p. 2019.

Shepherd, A. J., R. Swanepoel, and D. E. G., (1988). 'Evaluation of enzyme-linked immunosorbent assay and reversed passive hemagglutination for detection of Crimean-Congo hemorrhagic fevers virus antigen', Journal of Clinical Microbiology, 26(2), pp. 347–353.

Simon, M., C. Johansson and A. Mirazimi, (2009). 'Crimean-Congo hemorrhagic fever virus entry and replication is clathrin-, pH- and cholesteroldependent', Journal of General Virology, 90(1), pp. 210–215. doi: 10.1099/vir.0.006387-0.

Simuunza, M., W Weir, E. Courcier, A. Taita, B. Shielsa, (2011). 'Epidemiological analysis of tick-borne diseases in Zambia', Veterinary Parasitology, 175(3–4), pp. 331–342. doi: 10.1016/j.vetpar.2010.09.027.

Sorvillo, T. E., S. E. Rodriguez, P. Hudson, M. Carey, L. L. Rodriguez, C. F. Spiropoulou, B. H. Bird, J. R. Spengler and D. A. Bente, (2020).'Towards a Sustainable One Health Approach to Crimean – Congo Hemorrhagic Fever Prevention : Focus Areas and Gaps in Knowledge', pp. 1–28.

Spengler, J. R., (2019). 'ScienceDirect Crimean-Congo hemorrhagic fever and expansion from endemic regions ´ ric Bergeron and Christina F Spiropoulou', pp. 70–78. doi: 10.1016/j.coviro.2018.12.002.

Spengler, J. R. and D. A. Bente, (2017). 'Crimean – Congo Hemorrhagic Fever in Spain — New Arrival', New England Journal of Medicine, 377(2), pp. 106–108. doi: 10.1056/NEJMp1707436.Crimean.

Spengler, J. R., É. Bergeron and P. E. Rollin, (2016). 'Seroepidemiological Studies of Crimean-Congo Hemorrhagic Fever Virus in Domestic and Wild Animals', PLoS Neglected Tropical Diseases, 10(1), pp. 1–28. doi: 10.1371/journal.pntd.0004210.

Telmadarraiy, Z., S. M. Ghiasi, M. Moradi, H. Vatandoost, M. R. Eshraghian, F. Faghihi, Z. Zarei, A. Haeri and S. Chinikar, (2010). 'A survey of Crimean-Congo haemorrhagic fever in livestock and ticks in Ardabil Province, Iran during 2004-2005', Scandinavian Journal of Infectious Diseases, 42(2), pp. 137–141. doi: 10.3109/00365540903362501.

Tipih, T. and F. J. Burt, (2020). 'Crimean – Congo Hemorrhagic Fever Virus : Advances in Vaccine Development', 9. doi: 10.1089/biores.2019.0057.

Tonbak, S., M. Aktas, K. Altay, A. K. Azkur, A. Kalkan, Y. Bolat, N. Dumanli and A. Ozdarendeli, (2006). 'Crimean-Congo Hemorrhagic Fever Virus : Genetic Analysis and Tick Survey in Turkey', 44(11), pp. 4120–4124. doi: 10.1128/JCM.00644-06.

Torell, R., B. Bruce and B. Kvasnicka, (2003). 'Methods of Determining Age of Cattle', Cattle Producer's Library, CL 712, pp. 1–3. Available at: https://www.unce.unr.edu/publications/files/ag/other/cl712.pdf.

Verma, R. and P. Khanna, (2011). 'Verma-2011-Crimean-Congo haemorrhagic fever_ A', pp. 589–591.

Vescio, F. M., L. Busani, L. Mughini-Gras, C. Khoury, L. Avellis, E. Taseva, G. Rezza and I. Christova, F. M. et al., (2012). 'Environmental correlates of crimean-congo haemorrhagic fever incidence in Bulgaria', BMC Public Health, 12(1), pp. 1–7. doi: 10.1186/1471-2458-12-1116.

Vincent, K., A. J. Dougill, D. D. Mkwambisi, T. Cull, L. C. Stringer and D. Chanika, (2014). 'Analysis of Existing Weather and Climate Information for Malawi', University of Leeds, (April), pp. 1–18.

Walker, A. R., A. Bouattour, and I.Horak, (2014). Ticks of Domestic Animals in Africa : a

Guide to Identification of Species.

Whitehouse, C. A., (2004). 'Crimean-Congo hemorrhagic fever', Antiviral Research, 64(3), pp. 145–160. doi: 10.1016/j.antiviral.2004.08.001.

World Health Organization -AFRO, (2010). Integrated disease surveillance and response in the African Region- Technical Guidelines.

World Health Organization -EMR, (2021). Weekly Epidemiological Monitor, World Health Organization .Cairo,Egypt. Available at: https://applications.emro.who.int/docs/EPI/2021/2224-4220-2021-1405-eng.pdf.

World Health Organization , (2018a). 'Introduction to Crimean-Congo Haemorrhagic Fever', WHO document, pp. 1–23. Available at: https://www.who.int/emergencies/diseases/crimean-congo-haemorrhagic-fever/introduction.pdf?ua=1.

World Health Organization , (2018b). 'Roadmap for Research and Product Development against Crimean-Congo Haemorrhagic Fever (CCHF)', pp. 1–11. Available at: https://www.who.int/blueprint/priority-diseases/key-action/cchf-draft-r-and-d-roadmap.pdf?ua=1.

Wichgers Schreur, P. J., R. Kormelink and J. Kortekaas, (2018). 'Genome packaging of the Bunyavirales', Current Opinion in Virology, 33(M), pp. 151–155. doi: 10.1016/j.coviro.2018.08.011.

World Health Organization, (2016). 'A research and development Blueprint for action to prevent epidemics - Plan Of Action May 2016', (May), p. 44. Available at: https://www.who.int/blueprint/about/r_d_blueprint_plan_of_action.pdf.

Wu T-S, J., M. Kagoli, J. J. Kaasbøll, and G.A. Bjune, (2018). 'Integrated disease surveillance and response (IDSR) in Malawi: Implementation gaps and challenges for timely alert', PLoS ONE, 13(11), pp. 1–19. doi: 10.1371/journal.pone.0200858.

Yesilbag, K., L. Aydin, E. Dincer, G. Alpay, A. O Girisgin, P. Tuncer, A. Ozkul, (2013). 'Tick survey and detection of Crimean-Congo hemorrhagic fever virus in tick species from a non-endemic area, South Marmara region, Turkey', Experimental and Applied Acarology, 60(2), pp. 253–261. doi: 10.1007/s10493-012-9642-x.

Zivcec, M., F. E. M. Scholte, C. F. Spiropoulou, J. R. Spengler and É. Bergeron M. et al., (2016). 'Molecular insights into Crimean-Congo hemorrhagic fever virus', Viruses, 8(4). doi: 10.3390/v8040106.

https://wehrmed.de/article/3466-haemorrhagic-fever-in-the-field.html. (retrieved on 19/07/2019)

https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.05_CCHF.pdf (retrieved on 5/01/2021)

https://www.cfsph.iastate.edu/Factsheets/pdfs/crimean_congo_hemorrhagic_fever.pdf. (2019). Crimean-Congo HemorrhagicFever Crimean-Congo Hemorrhagic Fever. 1–9.

CHAPTER EIGHT

8.0 APPENDIXES

Appendix I: Study questionnaire

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VETERINARY MEDICINE

(Department of Disease Control)

DIRECTORATE OF RESEARCH AND POSTGRADUATE STUDIES

Sero-epidemiology in cattle and molecular investigation in ticks of Crimean-Congo Hemorrhagic Fever Virus (CCHFV) in central Malawi

Survey/study Questionnaire

Respondent ID Number: …….

Introduction:

I, am Marvin Phonera, a Master of Science student in One Health Analytical Epidemiology (OAHE) at the University of Zambia, School of Veterinary Medicine. I am undertaking a research project entitled 'Sero-epidemiology in cattle and molecular investigation in ticks of Crimean-Congo Hemorrhagic Fever Virus (CCHFV) in central Malawi.'

CCHF is a zoonotic disease that is transmitted by ticks. It is fatal in humans, but animals do not show clinical signs of infection. The findings of this study will help to inform the disease control authorities to make appropriate control strategies to safeguard the public from this disease.

I am, therefore, requesting for your consent to take part in this study. The information collected will remain confidential and only the researcher will have access to your details. You will not be individually identified in any report or presentation that will be produced from information collected through this questionnaire or shared by other means. The process will

include asking you a series of questions in relation to your herd of cattle and its management. Also, the study will involve collecting blood samples from some of your cattle.

Respondent endorsement; …… Date; ……

Interviewers name; ……. Date; ……..

Part A: Demographic Data

Location and respondent's identification details

Village……. . Extension Planning Area……

Veterinary Station……Cluster #:…… District……

Altitude ……. GPS Coordinates……

What is the name of cattle herd owner? ……

What is the age of cattle herd owner? ☐

What is the sex of cattle herd owner?
(male/female) ☐

What is the marital Status of cattle herd owner?
(Single = 1; Married = 2, Widow/widower = 3; Divorced = 4) ☐

What is the highest attained level of education by the herd owner? ☐
(Primary =1; Secondary = 2; Tertiary = 3; None = 4)

How many members are in this household? ☐

Part B: Livestock keeping history and Management

In this section, you will be asked about your cattle herd history and how you manage it.

What was the origin of your cattle herd?

(Inherited = 1; Bought = 2; Given = 3; other = 4)

If the answer to question 7 is not inherited, when did the household start rearing cattle? Specify……

What is the main purpose of keeping this herd? (Multiple answers are allowed and mark in an appropriate box (es))

(Prestige ☐ Commercial ☐ Drought Power ☐ Manure ☐ Other ☐

If the answer is other, specify …….

What is the size of your herd of cattle?

What other livestock species are kept in this household?

(Goats = 1; Sheep = 2; Pigs = 3; Donkey = 3; Other = 4; None = 5)

Number of goats

Number of sheep

How are the animals fed?

(Grazing = 1; Cut and carry = 2; Other = 3)

If the answer to question 14 is grazing; are the cattle grazed in upland or lowland (dambo)?

(Upland = 1; lowland = 2; both = 3)

Part C: Tick data

Do you know what ticks are? ☐

(No/ Yes)

(If answer to question 16 is No, go to question 27)

If answer to question 16 is yes, do you observe ticks on your animals? ☐

(No/ Yes)

If the answer to question 17 is yes, what is the tick infestation level? ☐

(No ticks = 0; low infestation (1-5 ticks) = 1; moderate infestation (6-10 ticks) = 2; high infestation (>10) = 3)

(The data collector should inspect the tick infestation level of the herd in question)

On a scale of 0 to 3, how do you rate the importance of ticks in your livestock farming?

(Not important = 0; Less important = 1; important = 2; very important = 3)

Explain your answer:……

In your own understanding, are animal ticks significant to human health?

(Yes/ No)

Explain:……

……

Part D: Tick control/ prevention data

Do you practice any tick control measures on your herd?

(No/ Yes)

If the answer to question 21 is 'Yes', which method (s) do you use?

(Dipping = 1; Spraying = 2; Pour-on = 3; Removing with hands = 4; Other = 5)

If the answer is other (5), Specify

……

How often is/are the(se) method (s) used?

(Daily = 1; Weekly =2; Fortnightly = 3; Monthly = 4; annually = 5)

What acaricide do you use? …….

(if the respondent does not know the name of the acaricide, let them produce container of the acaricide and the interviewer record the name given on the container)

Do you wear protective clothes when herding or handling your cattle?

(No/ Yes)

Have you or any member of this household been bitten by a tick in the past two years?

(No/Yes)

Are you involved in slaughtering livestock?

(No/ Yes)

Have you or any member of this household had fever but the cause was not malaria or other known causes?

(No/ Yes)

Have you or any member of this house experienced epistasis or any bleeding not resulting from injury?

(No/Yes)

End of questions, thank you very much for sparing your time to answer these questions.