Sero-epidemiological investigation of Crimean-Congo hemorrhagic fever virus infection in humans and livestock in West Africa

by

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Abstract

Crimean-Congo hemorrhagic fever virus (CCHFV), the causative agent of Crimean-Congo hemorrhagic fever (CCHF), is a zoonotic, tick-borne pathogen endemic in parts of Africa, Asia, Middle East, and Europe. CCHF outbreaks pose a significant threat to public health with a high case fatality ratio of up to 40%. Treatment is primarily supportive and there is no vaccine available. Human infections are often subclinical and in severe cases can lead to hemorrhaging and death as the risk of being misdiagnosed is common. Animals are asymptomatic hosts of CCHFV and are vital in the transmission cycle involving the primary vector, *Hyalomma* ticks. Ruminants have been shown to play a major role in the natural transmission cycle of CCHF, suggesting these are suitable indicator animals for serological studies to assess risks of human infections. This study, therefore, aimed to investigate the seroprevalence and seroepidemiology of CCHFV in at-risk populations (humans and livestock) in endemic settings of West Africa – Nigeria and The Gambia. For studies at both locations, serum samples were tested for the presence of CCHFV antibodies against the viral nucleocapsid protein using a validated dual antigen enzyme linked immunosorbent assay kit.

A convenience sampling of 486 serum samples were collected from apparently healthy and febrile participants between August 2010 and March 2018 from three major regions in Nigeria to identify risk factors and potential endemicity. To investigate the risk of zoonotic transmission of CCHF, serum samples were collected from indigenous goats (n = 544), sheep (n = 474), and cattle (n = 399) including recording georeferenced coordinates in environmental settings of close human-livestock interactions from selected villages at various locations in The Gambia. The sampled livestock in The Gambia had no relationship to the humans sampled in Nigeria.

In Nigeria, results revealed an overall seroprevalence of 1.65% (8/486) in at-risk human populations. CCHF seroprevalence in The Gambia was observed at 13.6% (138/1,018) in small ruminants and 59.9% (239/399) in cattle; with the seroprevalence of sheep 18.8% (89/474) being almost twice that of goats 9.0% (49/544). Primary risk factors for CCHF infection in humans included age, gender, and febrile illness; and data suggests that manifestation of febrile symptoms among patients may be associated with CCHFV infection in endemic settings. The results support previous epidemiological studies conducted in Nigeria and Senegal that borders Gambia indicating serological evidence and possible endemicity of CCHFV infection within these countries, as

CCHF has been consistently observed within these regions. Further confirmatory testing is needed to state CCHF is endemic within the area. To our knowledge, this is the first CCHFV serological study of cattle and small ruminants in The Gambia and results were compared to previous studies conducted throughout West Africa including Senegal. Seroprevalence in small ruminants and cattle are within the range detected elsewhere in endemic settings in sub-Saharan Africa. These results suggest that CCHFV has potential endemicity in The Gambia and West Africa generally.

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Dedication

To my mother, Michelle. For your guidance, love and prayers allowed me to grow into the person I am today. It is by your strength this flower bloomed despite adversity.

Chapter 1 - Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease caused by the Crimean-Congo hemorrhagic fever virus (CCHFV) (Bente, Forrester et al. 2013). This zoonotic disease primarily causes asymptomatic infections in animals, but humans can become accidental hosts of the virus (Bente, Forrester et al. 2013). Symptoms in humans can occur after a short incubation period and include sudden onset of chills, high fever, headache, and abdominal pain with possible nausea, vomiting, and diarrhea (Duygu, Sari et al. 2018). Hemorrhagic signs in severe cases can develop and become potentially fatal (Duygu, Sari et al. 2018). Human cases often are subclinical and run the risk of being misdiagnosed. An error that introduces the risk of nosocomial infections should hospitalization be required. With a high case fatality ratio of up to 40% and an estimated 10,000 – 15,000 human infections per year, the spread of CCHF from endemic to non-endemic areas is of great concern ((WHO) 2017). As treatment is primarily supportive and no vaccine is available, CCHF poses a significant threat to public health. Occupations that involve high human-livestock interaction are of particular concern - farmers, abattoir workers, traders, etc.

Hyalomma ticks act as the primary reservoir and vector of CCHF (Duygu, Sari et al. 2018). These ticks have a wide geographical distribution that parallel to the global distribution of CCHF (Duygu, Sari et al. 2018). Areas once believed to be CCHF-free are reporting cases in countries as far west as Spain (Spengler 2017) (Figure 1). The spread of CCHF can be attributed to a variety of factors: climate change, avian migration, livestock movement, or introduction of new hosts (Samudzi, Leman et al. 2012). CCHF emergence and re-emergence in endemic and non-endemic areas provides rationale to the importance of human and animal surveillance (Samudzi, Leman et al. 2012).

Animal seroprevalence studies provide useful data in determining the risk factors and level of disease risk in various geographic regions. This data can assist in determining the endemicity of the disease by establishing whether CCHF is consistently present within particular regions and offer insight to the potential risk of exposure for humans. Since CCHF is capable of infecting humans and various animal species, ideally a multi-species assay with quick and reliable results is best for CCHF surveillance (Sas, Comtet et al. 2018). A validated multi-species dual antigen enzyme linked immunosorbent assay (DA ELISA) developed based on the recombinant nucleoprotein of CCHFV is particularly suited to conducting serological investigation of potential virus infection or exposure in at-risk populations (Sas, Comtet et al. 2018). This DA ELISA utilizes the recombinant nucleocapsid protein derived from the African CCHFV strain IbAr10200 as diagnostic antigen. This strain belongs to clade III genotype of CCHFV, and the conservation of its nucleoprotein sequence was suggested to provide the advantage of identifying the additional CCHFV clades (Sas et al., 2018).

The objective of this study was to use the validated multi-species CCHF DA ELISA kits to detect the presence of anti-CCHFV antibodies in three population groups: A) at-risk healthy and febrile human populations in Nigeria, B) small ruminant populations at various localities and villages in The Gambia and C) cattle populations at various herds and villages in The Gambia. Prior studies established CCHF may be endemic in Nigeria and a probable cause of febrile illness. Based on this, the study in Nigeria investigated CCHF seroprevalence and to identify potential risk factors in humans. High CCHF seropositivity in animals has been suggested by previous studies to indicate CCHFV circulation in Senegal. As Senegal completely borders The Gambia, the livestock study aimed to investigate if CCHF was circulating in The Gambia.

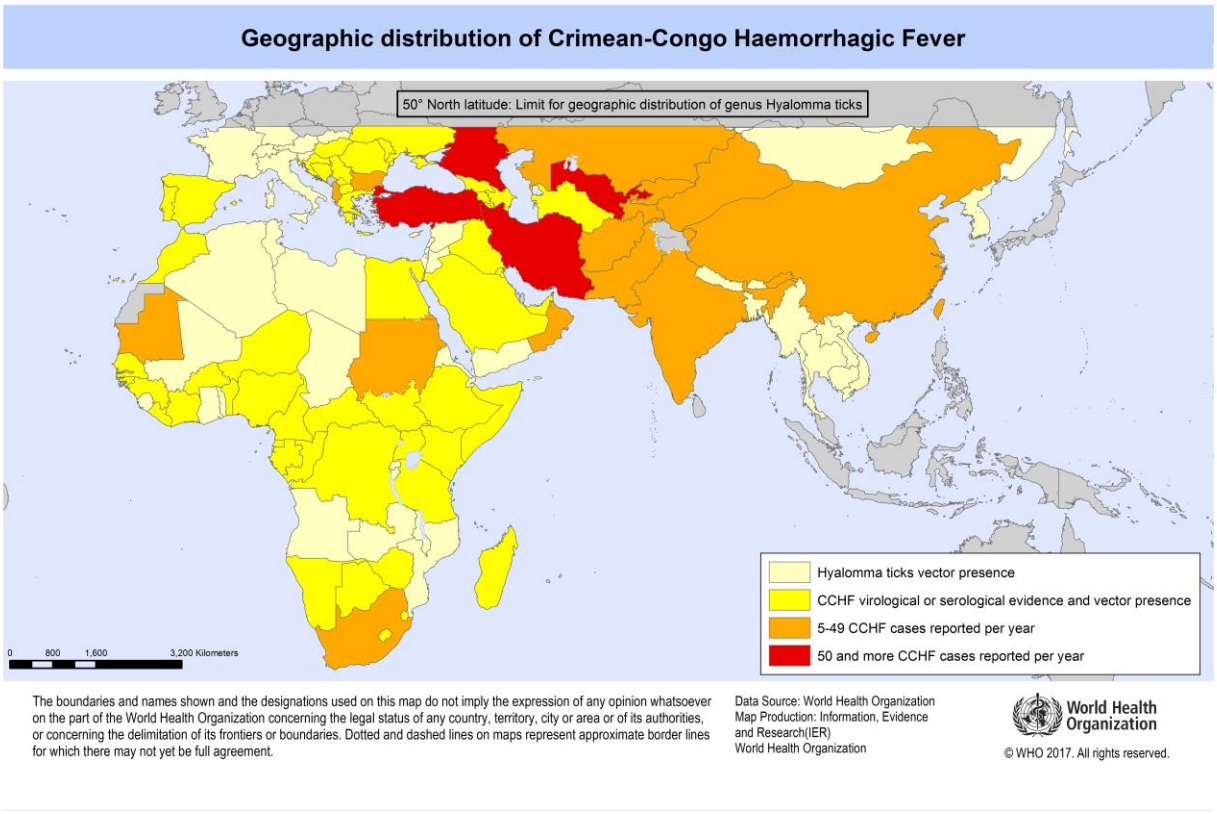

Figure 1. Map depicting the geographic distribution of CCHF and *Hyalomma* ticks' presence. Countries in light yellow indicate Hyalomma tick vector presence and countries in bright yellow possess CCHF virological or serological evidence plus vector presence. The number of CCHF cases reported per year are indicated by orange (5 – 49) and red (50+). Reprinted from World Health Organization. Copyright (2017).

Chapter 2 - Literature Review

2.1 History

Crimean-Congo hemorrhagic fever virus (CCHFV) is the most widespread, tick-borne viral disease affecting humans throughout Africa, Asia, the Middle East, and Europe (Emmerich, Mika et al. 2018). It has been reported that the 12th century era of Tadzhikistan contained the first descriptions of a hemorrhagic disease that later became considered to be Crimean-Congo hemorrhagic fever (CCHF) (Hoogstraal 1979). While CCHF was known to indigenous peoples of Southern Uzbekistan for centuries, the first clinical cases were documented in Crimea in 1944 (Hoogstraal 1979). In 1968, the virus known to cause Crimea hemorrhagic fever was shown to antigenically resemble Congo virus isolated from a febrile patient in 1956 and this finding led to the common name of Crimean-Congo hemorrhagic fever virus (Hoogstraal 1979).

2.2 Classification and genomics

CCHFV belongs to the genus *Orthonairovirus* within the family *Nairoviridae* of order *Bunyavirales* (Spengler, Bente et al. 2018). This is a single-stranded, negative sense RNA virus containing a tri-segmented genome with small (S), medium (M) and large (L) segments. The S segment (~1.6 kb) is comparable in size to other viruses within the *Nairoviridae* family and encodes the nucleocapsid protein (N). In contrast, the M segment (~5.4 kb) and L segment (~12.1 kb) are significantly larger when compared to the other *Nairoviridae* viruses. The M segment encodes the envelope glycoproteins and L segment encodes the RNA polymerase (Zivcec, Scholte et al. 2016). CCHFV has a spherical virion around 80-100 nm in diameter, as depicted in Figure 2. The lipid envelope is studded with spikes made up of the glycoproteins, G_N and G_C, that allow the virion to bind to cellular receptors (Bente, Forrester et al. 2013). The exact details of how these glycoproteins function for viral attachment and fusion is not known. CCHFV entry is also not well

understood, as the cellular receptors used by the virus have not been identified (Zivcec, Scholte et al. 2016).

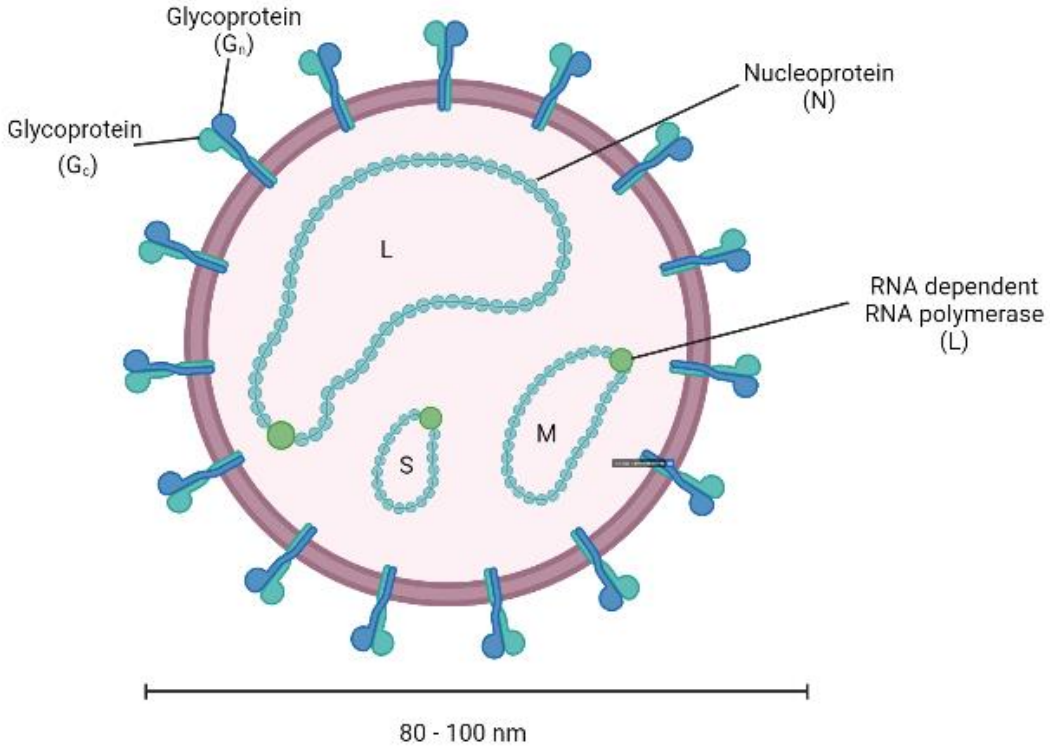

Figure 2. Virus structure of Crimean-Congo hemorrhagic fever virus

2.3 Phylogenetic relationship

Representation of CCHFV in sequence databases is poor due to limited availability of samples. Sequenced viruses provide an uneven representation of geographic spread as sample coverage is better in Europe and Asia over Africa (Lukashev, Klimentov et al. 2016). The majority of sequence data available are obtained from severe cases of human disease, sequence data of CCHFV in tick vectors and animal reservoirs remains minimal (Wampande, Waiswa et al. 2021). Despite limitations, studies have been able to compare available sequence data to group CCHFV

virus strains together into lineages that cover large geographic distances (Deyde, Khristova et al. 2006). There are seven CCHFV genotypes, defined by a distinct geographic region: group I - West Africa, II - Democratic Republic of the Congo; III - South Africa and West Africa, IV - Asia and Middle East, V - Europe and Turkey, VI - Greece; and VII - Mauritania (Deyde, Khristova et al. 2006, Wampande, Waiswa et al. 2021). This large geographic distribution of strains is suggested to be due to the movement of CCHFV infected livestock, movement of uninfected livestock carrying infected ticks, and migratory birds carrying infected ticks over large distances (Deyde, Khristova et al. 2006, Oklu M. 2020). Such animal movement may have created broad genetic diversity through introduction of multiple virus lineages (Burt, Paweska et al. 2009).

To determine the phylogenetic relationship between strains multiple studies have used sequence data and analyzed the S, M and L segments of CCHFV (Burt and Swanepoel 2005, Burt, Paweska et al. 2009, Sahay, Shete et al. 2021). Researchers examined 70 CCHF isolates gathered from 15 countries throughout southern, central and West Africa, Middle East, and Greece to sequence data from the S segment for phylogenetic analysis (Burt and Swanepoel 2005). The study broke down the tree topology into three distinct groups: A) an African clade with a predominantly Asian clade, B) southern and West Africa and C) a single virus isolated from Greece (Burt and Swanepoel 2005). Analysis determined up to 18% differences in nucleotide sequence between groups A and B, suggesting that there may be a slow exchange of genetic viral material between land masses as the two CCHFV lineages of A were more closely related to each other than that of B (Burt and Swanepoel 2005). To determine whether or not reassortment plays a role in CCHFV pathogenicity requires analysis of the M and L segments, as segmented RNA viruses have been shown to reassort during dual infections in nature (Burt, Paweska et al. 2009). Analysis of two Pakistan and 21 South African isolates using partial sequence data for the S, M, and L segments

and two regions of the M segment confirmed incongruencies were due to segment reassortment (Burt, Paweska et al. 2009). Analysis showed no incongruencies in the S and L segment data however, incongruencies were shown in the M segment for 15 of the South African and one Asian isolate suggesting virus movement between the two continents (Burt, Paweska et al. 2009). A recent study determined, through next generation sequencing (NGS), that reassorted Asian-West African genotype strains of CCHFV circulate in humans and ticks (Sahay, Shete et al. 2021). In that study, 29 clinical specimens and five tick pools were sequenced and mapped to S segment, Asian M segment, African M segment and L segment (Sahay, Shete et al. 2021). Researchers observed about 9% difference in nucleotides between S and L segments of the Asian sequences and found the M segment sequences of West Africa and two from India shared a common ancestor, thus concluding occurrence of reassortment within India (Sahay, Shete et al. 2021). Sequence data has improved our knowledge on CCHFV clades and can provide information on infection source within endemic areas, especially when contact tracing the path of spread in cases (Appannanavar and Mishra 2011).

2.4 Disease Ecology and transmission

The virus is maintained in nature through transovarial and transstadial transmission in over 30 species of Ixodid (hard) ticks that act as both vectors and reservoirs for CCHFV. Ticks within the genus *Hyalomma* are the primary vector but, other Ixodid ticks belonging to genera *Rhipicephalus*, *Dermacentor* and *Ixodes* are capable of transmitting the virus (Appannanavar and Mishra 2011). For transmission to occur, the virus must replicate in the midgut of the tick after being acquired from an infected bloodmeal. The virus spreads to the hemocoel, enters the salivary glands, and is transmitted to the next host via the tick's saliva (Gargili, Estrada-Pena et al. 2017). CCHFV occurs in both enzootic and epizootic cycles (Figure 3) (Hoogstraal 1979). Adult ticks

feed mainly on large herbivores and can transovarially infect immature ticks or transmit the virus transstadially if co-feeding with an immature tick on an infected host (Spengler, Estrada-Pena et al. 2016). CCHFV-infected immature ticks feed and infect small herbivores, such as hares, hedgehogs and some avian species that amplify the virus (Causey, Kemp et al. 1970, Hoogstraal 1979, Maltezou and Papa 2010, Spengler, Bergeron et al. 2016). The greater prevalence of CCHFV observed among large herbivores and may be due to the amplification limitations during the immature stages (Maltezou and Papa 2010). Infected nymphs emerging from infected, fed larva are only capable of infecting the same host the larva fed upon previously. A host fed on by multiple *Hyalomma* ticks of different life stages enables maintenance of CCHFV within an area (Maltezou and Papa 2010). Unlike other hemorrhagic fever viruses, CCHFV has a wide geographic distribution that overlaps with the distribution of *Hyalomma* ticks, limited to 50° North latitude (Maltezou and Papa 2010, Estrada-Pena, Ruiz-Fons et al. 2013). While the establishment of CCHFV is possible in nonendemic regions, this requires a variety of factors to be met: proper environmental and climate, movement of animal hosts and infected vectors, and human behavior that allows a high level of contact between vectors and appropriate hosts (Maltezou and Papa 2010). The diverse geographical regions *Hyalomma* ticks can exist in, including savannah, steppe, semi-desert, farmland, and grasslands, allows for the interactions of this tick species with a wide variety of animal species and humans (Mertens, Schmidt et al. 2013).

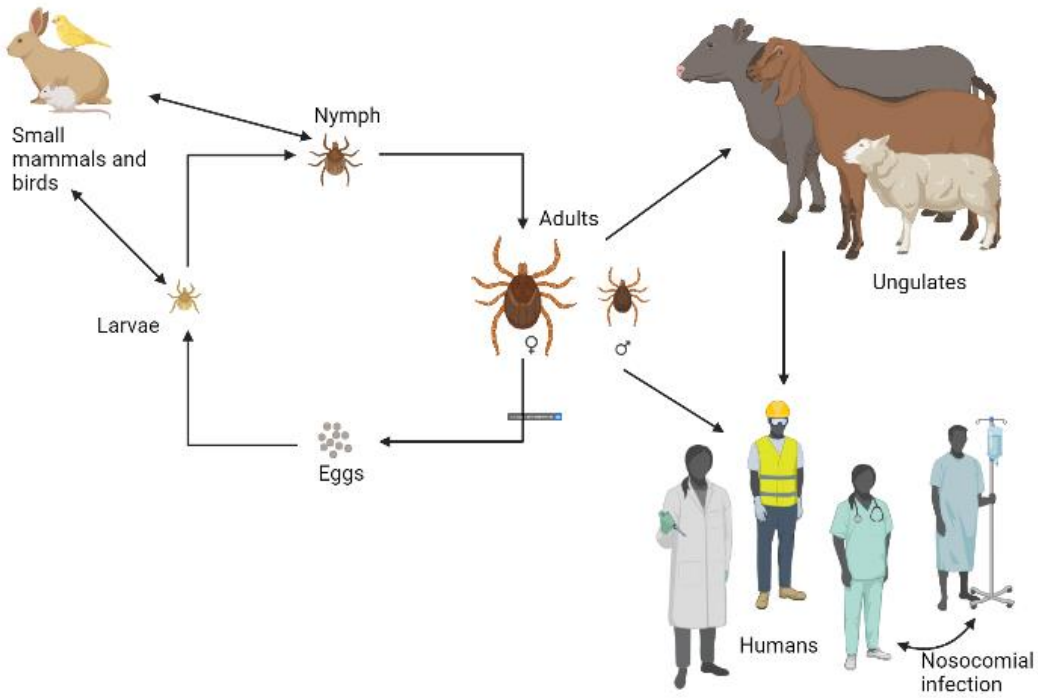

Figure 3. Transmission life cycle of CCHFV

2.5 Seroepidemiology and geographic distribution of CCHF

Between 1953-2010, over 6,000 cases of human CCHFV infection were reported from countries located in Southeastern Europe, primarily from Albania, Bulgaria, Greece, Kosovo and Turkey (Mertens, Schmidt et al. 2013). By 2010, CCHFV was detected in ticks collected from Spain and the first human cases began appearing in this country in 2016 (Spengler, Bente et al. 2018). A systematic review of 206 CCHFV seroprevalence studies conducted by Nasirian 2019 found the mean CCHFV seroprevalence in humans of 4.7% and 24.6% in animals. CCHFV seroprevalence can vary based on human occupation or animal species. As we work to better understand the ecology of CCHFV, domestic and wild animals have become another area of focus for sero-epidemiological studies (Figure 4).

CCHFV is enzootic within Africa, spreading throughout the continent from Egypt to South Africa with limited sero-epidemiological data available (Hoogstraal 1979). Several CCHFV seroprevalence studies in Africa are presented in the following paragraphs. As CCHF cases may go unnoticed in a country, despite having awareness of *Hyalomma* tick vectors being present, there is an important need to identify circulating virus strains circulating within at-risk areas for potential exposure (Aradaib, Erickson et al. 2010). Typically, cases of CCHF may go undetected or undiagnosed until severe human cases are involved.

A nosocomial outbreak of CCHF in Sudan led to the confirmation that CCHFV was indeed present in the country (Aradaib, Erickson et al. 2010). It was previously believed to not be circulating in Sudan as there were no confirmed CCHF cases but recent years have shown reports of suspect CCHF outbreaks and cases within the Kordufan region of Sudan (Aradaib, Erickson et al. 2010). Further confirmation stemmed from the finding of CCHFV specific antibodies present in camels, sheep and goats that were imported into Egypt and Saudi Arabia from Sudan (Aradaib, Erickson et al. 2010). From 1986-1987 in Egypt, researchers collected samples from 3,802 camels imported from Sudan and 499 from Kenya, to determine if prior CCHFV infection had occurred. Additionally, 600 indigenous sheep and cows were tested, and no presence of CCHFV antibody was detected except in camels. CCHFV antibody presence was observed in 14% of imported camels but no further transmission evidence of CCHFV among indigenous animals was observed (Morrill, Soliman et al. 1990). A recent study in Egypt (Chisholm, Dueger et al. 2012) found similar results, as researchers collected ticks from 43 freshly slaughtered cattle, buffalo, and sheep from livestock native to Egypt and camels imported from Sudan and Somalia. Of 138 tick pools tested, six were positive for CCHFV. While roughly 37.5% of the livestock were infested with ticks, none harbored any ticks that were positive for CCHFV. The six positive pools came from

ticks collected from one camel that was imported from Somalia and four camels imported from Sudan. Despite tracing the ticks to these animals, whether the infected ticks were imported into Egypt or acquired post arrival is unclear.

CCHF was observed in abattoir workers in Ghana. *Ambylomma* and *Hyalomma* ticks were collected from 57 of the 428 freshly slaughtered cattle, goats, and sheep. Five pools of ticks collected from cattle were positive for CCHFV. The overall seroprevalence of CCHFV among slaughterhouse workers was 5.7% (Akuffo, Brandful et al. 2016). The study identified the tick vectors of CCHFV in Ghana and the high exposure risks present to abattoir workers. Occupational health risks are high for employees of slaughterhouses, farms, butcher shops, veterinary clinics, and health care services. Daily routines for these workers can involve contact with infected animal carcasses, secretions, or tick bites that allow modes of CCHFV transmission into humans (Mostafavi, Pourhossein et al. 2017). According to Nasirian (2019), CCHFV seroprevalence is almost 7.5 times greater in at-risk professional populations than normal human populations. When comparing seroprevalence within animal populations on a global scale, these values average five times greater than normal human populations.

Available CCHFV data in Kenya is limited, making it difficult to say when and how CCHFV circulation occurred (Hoogstraal 1979). The 1960s had several human and bovine illnesses with hemorrhagic syndrome associations but whether that was due to CCHFV infection is uncertain. CCHFV detection was more certain in the 1970s, as the virus was isolated in *Rhipicephalus pulchellus* ticks collected from a dying sheep at the Kabete laboratory (Hoogstraal 1979). The first documented human case of CCHF occurred in 2000 when a young male farmer with acute hemorrhagic illness sought medical care in western Kenya. With viral hemorrhagic fever being suspected, the patient was isolated, and protocols implemented to avoid nosocomial

transmission. The patient passed away six days after developing symptoms, two days after admission, and serum samples were sent to the Arbovirus and Viral Hemorrhagic Fever Reference Laboratory for initial diagnostic testing (Dunster, Dunster et al. 2002). The facility lacked BSL-4 capacity to perform virus isolation but received positive results for CCHFV via RT-PCR and negative results for CCHFV specific antibodies. CCHFV presence was confirmed by sequencing of the RT-PCR amplicon at the Special Pathogens Unit in Johannesburg (Dunster, Dunster et al. 2002). Despite the findings of this study, CCHFV transmission remains understudied amongst human populations in Kenya.

A study in Kenya conducted from October 2010 through March 2011, evaluated serum samples from 517 acute febrile illness patients living within a pastoral, rural community to identify the prevalence of CCHFV specific antibodies using a commercial ELISA kit (VectorBest; VectorCrimean-CHF-IgG and IgM) (Lwande, Irura et al. 2012). Results showed the overall CCHF IgG seroprevalence of 19% (96/517), with only one patient testing positive for IgM antibodies (Lwande, Irura et al. 2012). The CCHFV seroprevalence was positively associated with age, with 36% of 40-49 years old suggesting seropositive. Greater seroprevalence in this age group may be due to these individuals being more active in animal handling or their increased need to assist family members by offering protection during seasonal migrations (Lwande, Irura et al. 2012). The risk of occupational exposure was greatest amongst farmers (29%), followed by housewives (18%). These findings are consistent with previous studies, as farmers interact closely with their livestock and housewives tend to care for smaller livestock, thus increasing the risk for contact with infected animals or infected ticks (Lwande, Irura et al. 2012).

CCHFV (previously Congo virus at the time the below studies began) surveillance was conducted in Nigeria from July 1964 to December 1968, with virus isolated from domestic

livestock, an African hedgehog, ticks, and *Culicoides* species (Causey, Kemp et al. 1969, Causey, Kemp et al. 1970). Blood samples were collected in Ibadan from febrile children, domestic animals within selected abattoirs or herds, and trapped wild animals and avian species. Additional serum samples were collected from survey participants and hospital patients (Causey, Kemp et al. 1970). Virus isolation required inoculation of infant mice and identification was performed using complement fixation (CF) and virus neutralization (VN). CCHFV was identified from a total of 34 samples: one goat, four cattle, one hedgehog liver/spleen, one pool of *Culicoides* and 27 from ticks of various genera. The CCHFV positive tick pools were primarily ticks collected off cattle and sheep. One positive tick pool was collected from a camel. While sampling occurred predominantly in Ibadan, there were some additional sampling locations included: Upper Ogun, Dada, Kano, Sokoto (central, southern or northern Nigeria) (Causey, Kemp et al. 1970). This study was important in contributing to the of understanding of the epidemiology of CCHFV. The findings suggest that the virus may be maintained within small mammals, the possibility of a tick-large animal cycle and possible ways domestic animals could be infected. This study highlighted the need for further research and development, including the roles of humans, domestic animals, and wildlife - especially migratory birds - may impact virus spread as well (Causey, Kemp et al. 1970).

Within sub-Saharan Africa, CCHFV epidemiology is poorly understood, and virus prevalence is unknown in many areas. The first serological study to investigate CCHFV prevalence occurred in Mozambique in 2015 (Muianga, Watson et al. 2017). Researchers collected serum samples from 300 febrile patients between March 2015 to March 2016 and tested for anti-CCHFV IgG antibodies using a commercialized ELISA kit (Vectocrimean-CHF IgG). The aim of that study was to determine if febrile patients were previously exposed to CCHFV. Results showed an overall

seroprevalence of 2.7% (8/300), suggesting that despite Mozambique being considered to lack any suspect or confirmed CCHF cases, the virus may be circulating within the country unknowingly (Muianga, Watson et al. 2017). Identification of seropositive individuals outside of known endemic areas is concerning because neighboring countries, including South Africa, are known to have high numbers of CCHF cases with outbreaks reported both in humans and animals (Burt F.J. 2007, Vawda, Goedhals et al. 2018). Mozambique shares borders with South Africa and the two countries conduct a substantial amount of commercial trade, including livestock trade (Muianga, Watson et al. 2017). Limitations on data availability makes it difficult for Mozambique to establish a proper surveillance program to assist in the preparation of prevention and control protocols for CCHFV.

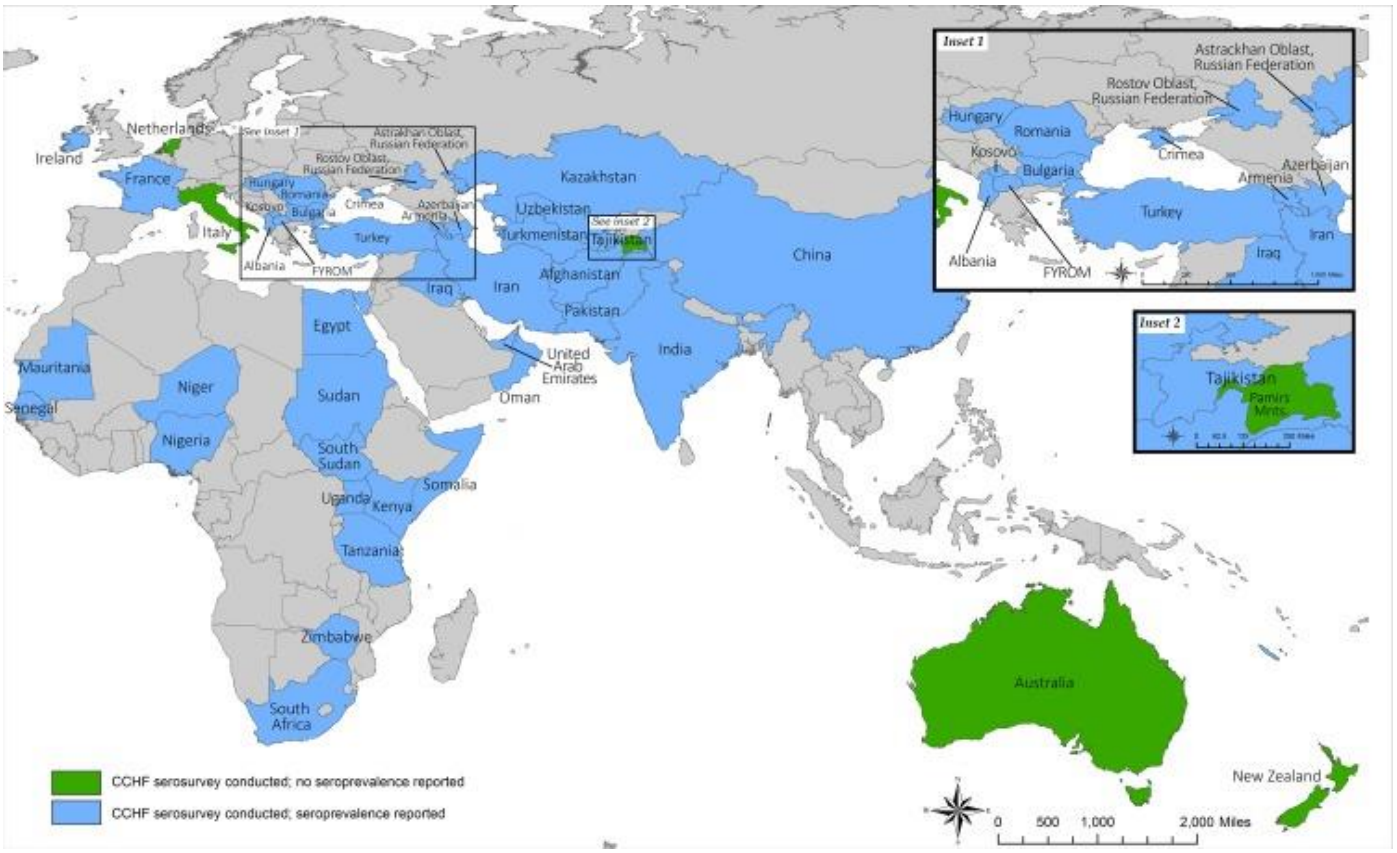

Figure 4. Geographic summary of countries represented in CCHFV sero-epidemiological surveys for domestic and wild animals. Countries in blue have evidence of seroprevalence in animals, countries in green lack seroprevalence, and countries in gray have no reported serosurveys. Reprinted from "Seroepidemiological Studies of Crimean-Congo Hemorrhagic Fever Virus in Domestic and Wild Animals", by Jessica R. Spengler, Eric Bergeron, Pierre E. Rollin, 2016, *Plos Neglected Tropical Diseases*, 10(1). Copyright (2016) by *Plos Neglected Tropical Diseases*

2.6 Clinical manifestation and pathogenesis

At-risk occupations for CCHFV include farmers, veterinarians and abattoir workers due to exposure to infected blood or ticks (Meurens, Dunoyer et al. 2021). Additional CCHFV high risk activities include improper use of proper protective equipment (PPE), livestock transportation, animal husbandry, and hunting (Nasirian 2019). Wet markets and hunting bushmeat increase the risk of CCHFV animal to human transmission from infected wildlife (Fisher-Hoch, McCormick et al. 1992, Meurens, Dunoyer et al. 2021). Primates and carnivores have been noted as the high-

risk species for CCHFV exposure in individuals that reported handling these wildlife species (Evans, Myat et al. 2021). CCHFV seroprevalence in human populations ranges between 0.1 – 14.4%, with higher seroprevalence rates, 16.5 – 30.3%, observed in people with at-risk occupations (Nasirian 2019). The highest reported CCHFV seroprevalence rates observed in at-risk professionals were: farmers (36.5%), animal workers (30.3%), and abattoir workers (16.5%) (Nasirian 2019).

Case fatality rates of CCHFV within humans are reported to range from five percent to upwards of 80% (Spengler, Bente et al. 2018). Despite high case fatality rates, most CCHFV infection cases are asymptomatic or result in a febrile illness not requiring hospitalization (Spengler, Bente et al. 2018). CCHFV infection involves four distinct phases: incubation period, pre-hemorrhagic phase, hemorrhagic phase, and convalescent phase. On average, of patients that require hospitalization, 90% of patients present with fever and hemorrhage (Spengler, Bente et al. 2018). The incubation period lasts three to seven days, while the pre-hemorrhagic phase lasts four to five days and progresses to the hemorrhagic phase in majority of patients. Major symptoms during the pre-hemorrhagic phase include high fever, headache, myalgia, nausea, abdominal pain, and non-bloody diarrhea. Typically, the hemorrhagic phase is short with rapid development of progressive hemorrhage. While the process of how CCHFV distributes throughout the body is not understood, animal models have shown the virus replicates first in the blood, liver, and spleen before spreading throughout the lungs, kidney and brain (Akinci, Bodur et al. 2013). Severe cases can lead to death as a result of multiorgan failure, disseminated intravascular coagulation and circulatory shock (Appannanavar and Mishra 2011). The convalescent phase, in survivors, begins 10-20 days post onset of symptoms (Appannanavar and Mishra 2011). Of all possible hosts for CCHFV, humans are the only species that develop clinical disease manifestations (Appannanavar

and Mishra 2011). In comparison, animals infected with CCHFV tend to exhibit asymptomatic infections (el-Azazy and Scrimgeour 1997). Once infected, small mammals undergo a short viremic period of 2-15 days before developing anti-CCHFV antibodies (Spengler, Estrada-Pena et al. 2016).

CCHF pathogenesis is poorly understood as complete autopsies rarely occur on patients with fatal outcomes due to CCHF infection (Whitehouse 2004). Limited laboratory capacities and lack of animal models makes studying this aspect of the disease difficult. Previous observations of dysregulated host responses caused by hemorrhagic fever viruses infecting immune cells has led to speculation that virus interaction with host cells, particularly endothelial cells and immune cells, are the cause of pathogenesis of CCHF (Akinci, Bodur et al. 2013, Rodriguez, Hawman et al. 2022). Dysregulated inflammatory responses have been shown to contribute to severe mortality and morbidity in other hemorrhagic fevers but knowledge on if this occurs in CCHF is lacking (Rodriguez, Hawman et al. 2022). Through directly effecting immune cells and inducing cell injury via inflammatory pathways, hemorrhagic fever viruses have managed to modify hemostasis ((Chen and Cosgriff 2000) as cited in (Akinci, Bodur et al. 2013)). Making damages to the endothelium and immune response appear vital to clinical progression and disease severity in CCHF infections (Akinci, Bodur et al. 2013). These mechanisms combined with factors such as disseminated intravascular coagulation, liver dysfunction, and thrombocytopenia can lead to hemorrhaging in severe cases (Chen and Cosgriff (2000) as cited in (Akinci, Bodur et al. 2013)).

2.7 Detection and testing options

Early detection of CCHFV infection is pivotal to avoid further transmission, particularly within nosocomial settings. Suspect CCHF cases occur when an acutely ill individual has known

exposure concerns due to ticks, infected animal blood or tissues, or interaction with a confirmed human CCHF case (Bente, Forrester et al. 2013). Overall testing is time sensitive depending on the available test method(s). Humans tend to be viremic at days 7-10 post infection (Bente, Forrester et al. 2013). There are multiple assays capable of detecting CCHFV infection at various stages of disease but diagnostic assay choice may vary based on assay type and biosafety level (BSL) requirements which are not consistent across many countries (Raabe 2020). Nucleic acid amplification tests (i.e. reverse transcriptase PCR), viral antigen detection assays and viral culture (via cell lines of monkeys, chickens and hamsters) are useful during the viremic period, the first week after onset of symptoms occurs (Raabe 2020). Serological testing is useful after the first week of illness, approximately seven to nine days after infection once seroconversion occurs (Raabe 2020).

The nucleocapsid (N) protein of CCHFV has been shown to induce long lasting immune responses in humans (Emmerich, Avsic-Zupanc et al. 2010). Combined with recent molecular technique advancements, the use of recombinant N protein in serological assays for diagnosis and sero-surveillance has become common (Emmerich, Avsic-Zupanc et al. 2010, Rangunwala, Samudzi et al. 2014). Detection of IgM and IgG antibodies against the recombinant N protein antigen within an enzyme-linked immunosorbent assay (ELISA) or immunofluorescence assay (IFA), allows for testing to occur within BSL-2 laboratories after sample inactivation (Fernandez-Garcia MD 2014). The presence of virus specific antibodies within the animal's serum allows for CCHFV exposure evaluation in livestock and wildlife. Many studies have relied on serological assays to investigate vertebrate host (including humans) exposure to CCHFV (Bente, Forrester et al. 2013). IgM antibodies can be detected from four days post onset of disease up to four months (Mertens, Schmidt et al. 2013). While IgG antibodies are detectable from day six after the

incubation period up to five years (Mertens, Schmidt et al. 2013). Commercially available IgG and IgM ELISAs are scarcely accessible to detect CCHFV specific antibodies in humans and animals due to difficulties in ordering for international customers, while there are several in-house ELISAs published for testing human sera with limited literature regarding the diagnostic sensitivity and specificity (Mertens, Schmidt et al. 2013). Lack of access to CCHFV diagnostic assay is problematic within many endemic countries. Improved access to these diagnostic assays, that could be used across both human and animal species, could help facilitate studies on how CCHFV circulates within endemic areas (Mertens, Schmidt et al. 2013).

Focus on further developing serological assays that are readily available would be useful, as these methods potentially cover more strains due to cross-reactivity and the concern would be to ensure the test sensitivity is not affected by variation amongst CCHFV strains (Fernandez-Garcia MD 2014). The double antigen ELISA validated in the Sas, Comtet et al. (2018) study found the assay to be highly sensitive and specific for antibodies against CCHFV while detecting across multiple clades without significant difference. Sensitivity was found to be 98.9% as 268/271 of the positive reference sera correctly tested positive and specificity at 100% with all negative reference sera being confirmed negative (Sas, Comtet et al. 2018). A sensitivity of 98.9% in this assay indicates that the assay could identify CCHFV antibodies in majority of samples but identified a few as positives falsely. Meaning the assay identified true positives, or the proportion of samples that are genuinely positive providing a positive result, at a high rate but also identified some negative CCHFV antibody samples incorrectly as false positives. The assay having a specificity of 100% indicates that true negatives, or proportion of samples being identified as negative containing CCHFV antibody negative serum, were correctly identified. While the assay identified a few false positive samples, there were no false negatives (Sas, Comtet et al. 2018).

The development of serological assays that are highly sensitive and specific allow serological testing to accurately determine if an exposure or infection may have occurred in an individual prior to further confirmatory testing. For surveillance purposes, the high sensitivity and specificity seen with this CCHF DA ELISA assay instills confidence that serum identified as negative for CCHFV antibodies may be a true negative and focus can be placed on ensuring positive results are true positives to implement confirmatory testing or public health measures based on exposure risk factors.

2.8 Treatment and control

Treatment of CCHF and prevention and control of CCHFV is difficult (Kouhpayeh 2021, Pandya and Rajput 2021). Currently, there is no approved vaccine readily available for CCHFV. An inactivated vaccine is used only in Bulgaria and the safety issues regarding the vaccine prevents its use in countries endemic for CCHFV (Mousavi-Jazi, Karlberg et al. 2012). An international collaboration was established in 2017 for the development of CCHFV vaccines through the CCHF Vaccine initiative (Hawman and Feldmann 2018). Coordinated by the Public Institute of Sweden, the six-year project focuses on the development of a vaccine that can control CCHFV globally (Public Institute of Sweden 2017). Difficulties in vaccine development is partly due to the high genetic variability between CCHFV strains and limited laboratory capacity of the appropriate biosafety level.

Treatment of CCHF is primarily supportive (Kouhpayeh 2021). In patients that develop severe clinical signs or hemorrhage, medical care can involve hematological treatments such as blood transfusions (Mertens, Schmidt et al. 2013). The use of antiviral treatment, namely ribavirin, remains controversial due to its efficacy and side effects (Rusnak 2011, Kouhpayeh 2021). Immunotherapy treatment options have been studied but there is limited data on the benefits of

anti-CCHFV immunoglobulin therapy (Appannanavar and Mishra 2011, Mertens, Schmidt et al. 2013).

Targeting CCHFV prevention at the community level and within nosocomial settings are the most effective routes to control the disease (Appannanavar and Mishra 2011). The endemic tick-animal transmission cycle is rarely noticed as animals remain asymptomatic, making changes in transmission intensity within an area difficult to monitor and prevent ((WHO) 2017). The World Health Organization (WHO) recommends community engagement through public health measures to reduce human infection and exposure risks. Strategies to reduce the risk of tick-to-human CCHFV transmission, animal-to-human transmission, and human-to-human transmission are described below ((WHO) 2017). Wearing proper protective equipment and clothing when handling animals or animal products would reduce risk, especially in slaughtering and butchering practices. Implementation of animal quarantine with testing prior to allowing new animals to enter herds reduces the risk of exposure to both humans and the herd (Sorvillo, Rodriguez et al. 2020, Meurens, Dunoyer et al. 2021). Humans can further minimize potential risks through wearing light colored, long sleeves/trousers to easily detect ticks on clothing, use of approved arthropod repellents, and avoiding tick-infested areas especially during seasons of high tick activity ((WHO) 2017). Risk reduction in human-to-human transmission will rely heavily on controlling nosocomial infections through implementing proper protocols in healthcare settings. Examples of methods to reduce nosocomial infections can include: use of proper protective equipment when caring for suspected or confirmed CCHF patients and specimens, basic hand hygiene practices, only allow properly trained staff to work with suspected CCHF cases, and have standard infection control precautions prepared prior to outbreaks ((WHO) 2017). While none of these methods are an absolute solution,

they collectively demonstrate the need for public health measures to properly educate the public about risks and protective measures for CCHF in endemic.

Chapter 3 - Crimean-Congo hemorrhagic fever and the seroprevalence of human infections in Nigeria

3.1 Introduction

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne virus that can lead to severe, potentially fatal hemorrhagic fever in humans (Sorvillo, Rodriguez et al. 2020). CCHFV was first recognized as a cause of human illness in the Crimea region of the former Soviet Union in 1944 and was referred to as Crimean hemorrhagic fever (CHF) (Bente, Forrester et al. 2013). In 1968, researchers found a viral strain of CHF isolated from a patient to be antigenically indistinguishable from Congo virus and the virus was renamed Crimean Congo hemorrhagic fever virus (Hoogstraal 1979). CCHF has high case fatality rates of 5 - 30% with supportive care being the main form of treatment (Zivcec, Scholte et al. 2016).

CCHFV has been identified in ticks in Nigeria since the 1970's and data on human infection is limited (Bukbuk, Dowall et al. 2016). Prior to CCHFV etiology being understood, Congo virus was believed to be the causative agent, most frequently isolated in Nigeria from domestic cattle, goats, an African hedgehog, and various tick species (Causey, Kemp et al. 1970, David-West, Cooke et al. 1974). Virus isolation attempts through the 1960's provided no evidence of Congo virus leading to human infections in Nigeria and researchers lacked survey data on Congo virus antibodies (Moore, Causey et al. 1975). A survey of neutralizing antibodies to Congo virus in Nigeria was able to be conducted from 1973 – 1974 (David-West, Cooke et al. 1974). David-West et al., 1974 detected antibodies to Congo virus in 24 out of the 250 (9.60%) febrile human sera tested and suggested Congo virus could be a threat to human health in Nigeria under favorable conditions.

Further evidence that humans in Nigeria can be exposed to CCHFV is a study by Bukbuk et al. 2016, where the first documented CCHFV human case within Nigeria was identified through direct whole genome sequencing of viral RNA in a human sample. Serologically, CCHFV antibodies among 1,189 human sera were investigated and seroprevalences of 10.6% IgG, 3.5% IgM, and 0.6% IgG+IgM were observed (Bukbuk, Dowall et al. 2016). Despite a 38-year time gap, the data from Bukbuk, Dowall et al. (2016) and David-West, Cooke et al. (1974) reported similar seroprevalence rates, and Bukbuk, Dowall et al. (2016) suggests a similar frequency of human CCHFV infection remains a potential cause of febrile illness in patients in Nigeria and warrants further investigation (Bukbuk, Dowall et al. 2016).

With previous studies establishing CCHF as potentially endemic in Nigeria and a probable cause of febrile illness, our study aimed to investigate the prevalence of CCHF at three sites in the country. Focus was placed on apparently healthy and febrile participants to identify associated risk factors in at-risk human populations.

3.2 Materials and Methods

Study sites

Abuja and Jos are centrally located within the country, possess cooler climates than Ibadan, and sit at greater elevations, 2,760 feet and ~ 4,000 feet above sea level respectively (Figure 5) (Abubakar 2014, Oluwole and Daful 2014). Ibadan has a lower average elevation around 738 feet above sea level and is located an estimated 100 miles from the Atlantic coast (Eguaroje, A et al. 2015). While all three cities are urbanized, Ibadan is heavily open market focused with locations on majority of street corners and Ibadan is in a highly vegetative area as the rainforest environment is dominant.

Humans and sampling collection

A convenience sampling of 486 human serum previously collected from apparently healthy and febrile participants in endemic settings took place within the Nigerian cities of Abuja (AB), Ibadan (IB), and Jos (PL). These samples were collected in 2011 (AB), 2017 (PL) and 2018 (IB) through a collaborative agreement with the Vom Veterinary Research Institute in Nigeria for an unrelated study. The samples tested in this study were those that still contained the required amount of serum needed per the DA ELISA kits. The participant questionnaire asked for information regarding their age, gender, occupation, history of animal exposure, presence of fever and to identify their exposure type based on occupational exposure or febrile patient.

Figure 5. Map of sampling locations in Nigeria

Dual antigen enzyme linked immunosorbent assay (DA ELISA)

ID.Vet (France) developed the ID Screen CCHF Double Antigen multi-species ELISA kits used in this study. The assay was conducted according to manufacturer instructions. Plates come precoated with recombinant CCHFV nucleoprotein antigen. To each well, 50 µl of dilution Buffer

14 (ID.Vet, France) was added to the precoated ELISA plate. Thirty microliters of positive control, negative control, or serum sample were added to their respective well, with all samples tested in duplicate. The plate was covered and incubated at 21°C (± 5°C) for 45 minutes. The plate was washed five times with 300 µl of wash buffer per well. To each well, 50 µl of the conjugate (1X) was added and the plate covered and incubated at 21°C (± 5°C) for 30 minutes. Afterwards, the plate was washed five times with 300 µl of wash buffer per well. To each well, 100 µl of Substrate Solution (ID.Vet, France) was added, the plate covered and incubated in the dark at 21°C (±5°C) for 15 minutes. The reaction was stopped by adding 100 µl of Stop Solution (ID.Vet, France) to each well (Figure 6).

The optical density (OD) was measured at 450 nm. Tests were considered validated if the mean OD_{450} of the positive control OD (OD_{PC}) was greater than 0.350 and the ratio of the mean values of the positive and negative controls (OD_{PC} and OD_{NC}) were greater than 3. Each sample was interpreted by calculating the sample to positive ratio (S/P) percentage:

$$S/P\,\% = \frac{OD_{SAMPLE}}{OD_{PC}} \times 100$$

Serum samples with a S/P percentage of less than or equal to 30% were considered negative and S/P percentages greater than 30% were considered positive. An analysis program provided from ID.Vet (IDSoft data analysis) was used for data analysis.

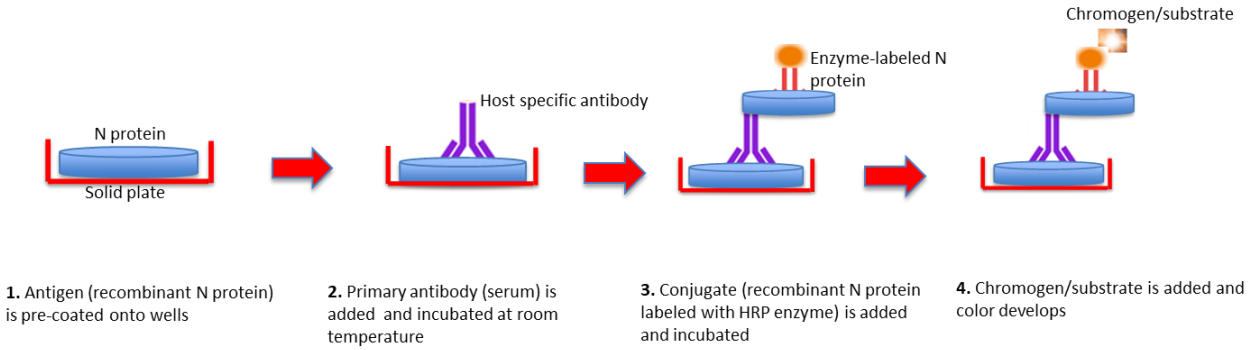

Figure 6. CCHF double-antigen ELISA procedure

3.3 Results

Of the 486 serum samples ran, 8 tested positive for presence of CCHFV antibodies giving an overall seroprevalence of 1.65% (8/486). Abuja accounted for 120/486 of samples (24.7%), Ibadan for 247/486 (50.8%) and Jos for 119/486 (24.5%) of samples (Figure 7). Seven of the CCHFV antibody positive samples originated from Ibadan and one from Abuja. There were no positive samples from Jos.

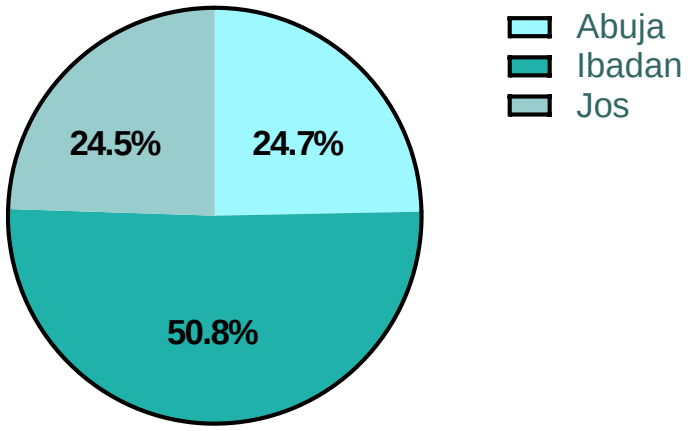

Figure 7. Percentage of sampling taken per sampling location. n = 486

From the administered questionnaire (Figure 8), a variety of occupations were represented among the CCHFV antibody positive cases: butcher (1), civil servant (1), farmer (1), pensioner (1), student (2), and trader (2). Only one case was a likely occupational exposure (AB193, butcher) based on the exposure the participant reported while the remaining were reported febrile patients. Likely animal exposure occurred for three of the positive cases (AB193, IB019, IB078). Fever was documented in all but one positive case (AB193), as shown in Figure 9 and Figure 10.

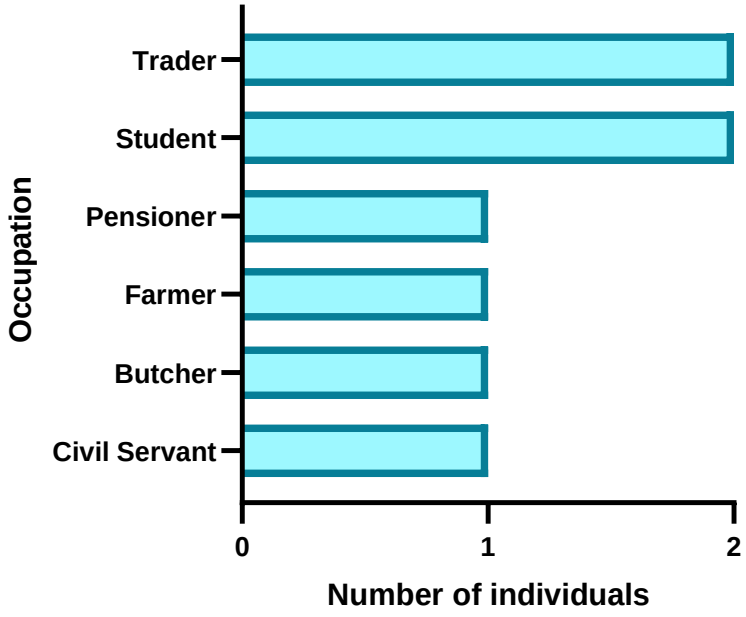

Figure 8. Distribution of seropositive cases by occupation

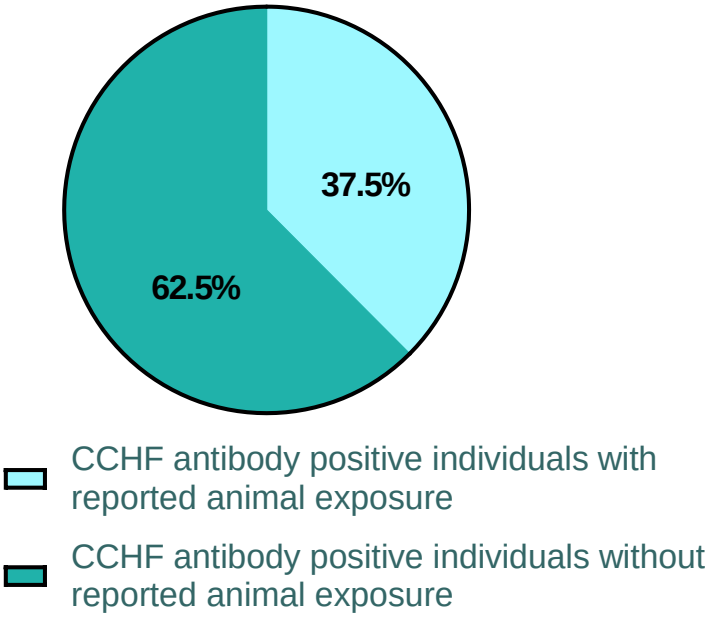

Figure 9. Percentage of CCHF antibody positive cases with reported animal exposure versus without reported animal exposure. Total number of CCHFV antibody positive individuals = 8

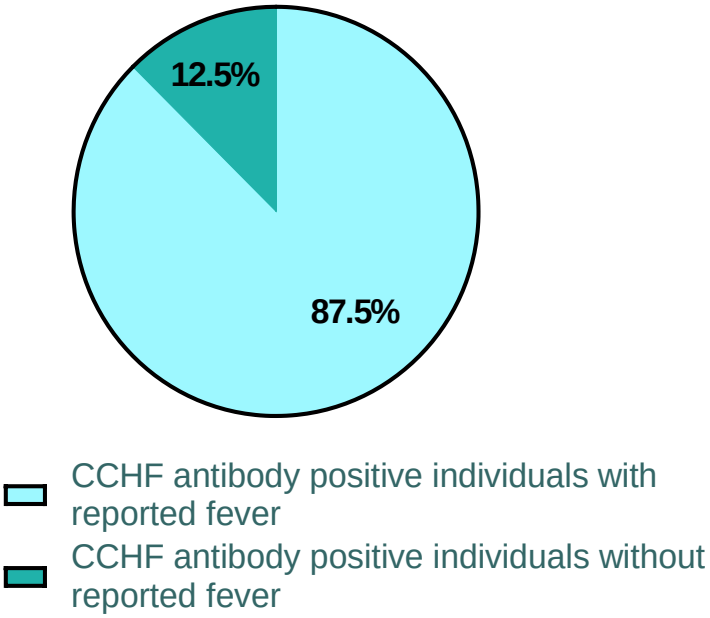

Figure 10. Percentage of CCHF antibody positive cases with reported fever versus without reported fever. Total number of CCHFV antibody positive individuals = 8

The CCHFV antibody positive cases ranged in age from 5 years to 78 years old. Of the positive cases, 50% (4/8) were between 40-80 years old (Figure 11). Positive cases among 1-19 years and 20-39 years each accounted for 25% (2/8) of the positive cases. Females accounted for 62.5% (5/8) of the antibody positive cases and males accounted for 37.5% (3/8) of positive cases (Figure 12). Table 1 depicts the specific details for each seropositive individual.

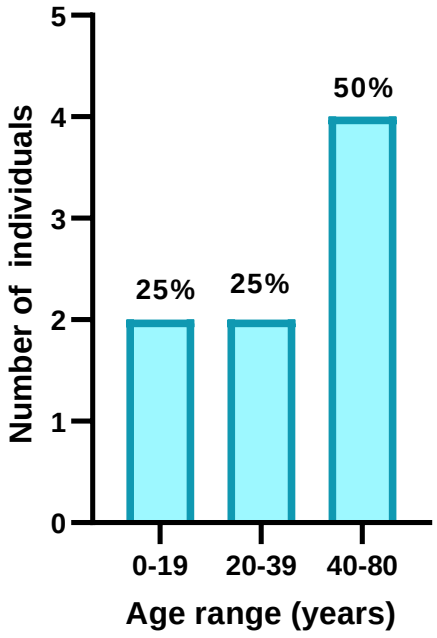

Figure 11. Distribution of age ranges based on CCHF antibody positive individuals

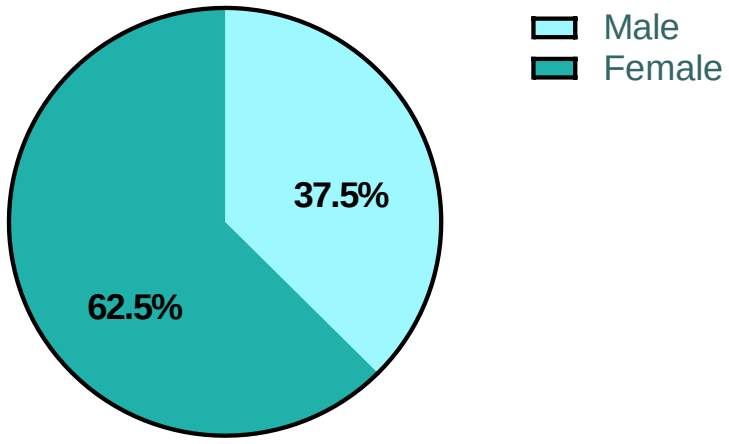

Figure 12. Percentage of CCHF antibody positive cases by gender. Total number of antibody positive individuals = 8

Table 1. Detailed information on each CCHF antibody positive case

Sample ID	Location	Sex	Age (years)	Occupation	Animal Exposure	Fever	Exposure type
AB 193	Abuja	Male	38	Butcher	Yes	No	Occupational
IB 019	Ibadan	Female	67	Trader	Yes	Yes	Febrile patient
IB 078	Ibadan	Male	78	Farmer	Yes	Yes	Febrile patient
IB 215	Ibadan	Female	30	Student	No	Yes	Febrile patient
IB 256	Ibadan	Female	71	Pensioner	No	Yes	Febrile patient
IB 272	Ibadan	Female	18	Student	No	Yes	Febrile patient
IB 276	Ibadan	Female	65	Civil Servant	No	Yes	Febrile patient
IB 298	Ibadan	Male	5	Trader	No	Yes	Febrile patient

3.4 Discussion

Previous seroprevalence studies (Causey, Kemp et al. 1970, Okorie 1991) conducted in Ibadan noted varying levels of CCHFV antibodies presented in both humans and animals. In this study, we have detected varying CCHF seroprevalence at the three study sites: a seroprevalence

of 2.83% (7/247) was detected in Ibadan, 0.83% (1/120) in Abuja and 0.0% (0/119) in Jos. The study sites are characterized by different climatic conditions and ecological systems that may present varying levels of risk factors for infection. Although the rainforest vegetation in Ibadan may support tick abundance, the relatively low seroprevalence of human infections and the lack of data on human tick infestations or tick bite made it impossible to establish the role tick transmission may have played in these infections. Meanwhile, the elevations of each site were Ibadan at 225 m above sea level, Abuja at 456 m above sea level and Jos at 1,265 m above sea level. Few studies have been conducted to examine the influence of altitude on CCHFV incidence and tick prevalence, but according to Zivalioglu (2008) as cited in Sisman (2013), 74% of CCHFV infected individuals reside 600 – 1200 m above sea level, averaging around 800 m. Further data from Sisman (2013) suggests this concept of altitude plays a role in CCHFV incidence. Results of the current study, however, did not seem to demonstrate or confirm this relationship.

The increased livestock movement within Ibadan adds to the human associated CCHFV risks. With easy road access, movement of livestock to and from these markets creates increased interaction and contact with livestock. This introduces possible routes of CCHFV animal-to-human and tick-to-human transmissions.

Human-to-human transmission could be a likely occurrence as many individuals work in areas with occupational exposure risk, such as traders and students. These occupations involve close human-to-human interaction. Other individuals exhibiting exposure belong to occupational categories such as farmers, butchers, and civil servants. Farmers, and most particularly butchers, face significantly high risk of exposure of CCHFV infection via close contact with livestock and infected carcasses/aerosol transmission, respectively. A prior study described risk of infection being highest in farmers or farm laborers, particularly those with direct contact with cattle, sheep

and goats (Fisher-Hoch, McCormick et al. 1992). The comparatively higher seroprevalence in Ibadan suggest that the site may be a risk area for CCHFV infection. Moreover, the farming capacities, and cooler climates of Abuja and Jos, with the latter possessing one of the coolest climates in Nigeria, may likely limit the spread of CCHF.

Due to the ease of CCHFV transmission from infected blood and tissues, occupational exposure is an area of concern for humans. Nosocomial settings can be a major risk, emphasizing the need for implementing control measures. Animal exposure was not a significant risk factor to the Nigerian populations within this study. However, this finding should be taken with caution as it is not unusual for individuals who visited animal kraals, abattoirs, or livestock markets to respond in the negative regarding any prior interaction with livestock. The three antibody positive cases with animal exposure were in occupations that we would anticipate being at risk for exposure: Farmer (IB078), Trader (IB019), and Butcher (AB193). These findings are consistent with the previously stated epidemiological knowledge that occupations in agriculture, slaughterhouses, and livestock trade have a higher potential risk for CCHFV exposure. The results of the study indicate that CCHFV may be the cause of febrile disease in patients. Based on the data, fever is associated with CCHF. This is significant information to consider when implementing public health measures for CCHF prevention and control in these endemic areas, particularly within Ibadan as all the antibody positive cases that reported having fever (87.5%, 7/8) were from this area. Health officials should be educated to consider CCHFV as a possible cause of febrile disease in Ibadan.

Nigerian society is patriarchal, and the presence of traditional gender roles especially in farming and trade presents disproportionate risk. Seventy-eight percent of women work within the informal sector as farmers, food processers, traders, and market vendors (Makama 2013). Knowing

the societal constructs of Nigeria, the higher number of women presenting with CCHFV antibodies is consistent within the results of this study. Interacting with the field, livestock, or marketplaces contributes to a potential higher risk of exposure through tick-to-human, animal-to-human, and human-to-human transmission. Markets are heavily congregated, with women and children commonly working in these areas, thus the CCHFV antibody positive case of the 5 years old male may have been exposed to infection via close-contact transmission.

The data suggests age is a risk factor for positive antibody serostatus as the majority of the antibody positive individuals were over 40 years old. Since immunity wanes as age increases, younger adults exposed to CCHFV could have a better primary immune response compared to older adults, over 40 years old, whose primary immunity is already compromised due to their age (Nikolich-Žugich 2014). Understanding the interactions between immune response during CCHFV infection within different age groups may influence treatment recommendations.

3.5 Conclusions

This study shows the need to continue seroprevalence studies and implement surveillance within human populations in Nigeria. Considering older individuals have a higher CCHFV seroprevalence, this is suggestive that most of the antibody positive cases were not due to recent exposure or infection in this location. Furthermore, studies conducted since the 1980's have regularly found CCHFV antibodies present in both humans and animals located in Nigeria, supports that CCHFV is likely established in Nigeria. Identifying febrile patients, older age individuals and women as being at higher risk for CCHFV infection provides a good basis to develop public health measures in these areas. Increasing our epidemiological knowledge with additional studies will help guide development of more comprehensive recommendations to reduce CCHFV associated risk factors. To understand the full extent of CCHFV risk and the epidemiology

in West Africa, further studies taking a OneHealth approach aimed at understanding the epidemiology and dynamics of infection in animals and tick vectors are highly necessary.

Chapter 4 - Seroprevalence of Crimean-Congo hemorrhagic fever in small ruminants of The Gambia

4.1 Introduction

Crimean Congo hemorrhagic fever virus (CCHFV) is a zoonotic, tick-borne viral infection that is maintained through vertical and horizontal transmission of several species of ixodid (hard) ticks capable of spreading the virus to a variety of wild and domestic animals (Bente, Forrester et al. 2013). Animal infections are asymptomatic, with viremia and CCHFV antibodies previously observed in cattle, sheep, goats, horses, camels, pigs, donkeys, mice, domestic dogs, hares, and ostriches (Mangombi, Roqueplo et al. 2020). Ruminants have been shown to play a major role in the natural transmission cycle of CCHFV, suggesting these are suitable indicator animals for serological studies to assess human risk for CCHFV infection (Hartlaub, Daodu et al. 2021). Researchers in Mauritania observed CCHFV seroprevalences of 16% in sheep and 15% in goats with antibody prevalence increasing in older age groups, suggesting increased age corresponds with higher risk of exposure to CCHFV positive ticks within endemic areas (Schulz, Barry et al. 2021). Seroprevalence values in goats and sheep have some variation between the different countries of West Africa. A study in Nigeria observed seroprevalence rates at 2.0% for goats and 0% in sheep when conducting an IgG-ELISA in all adult aged animals (Oluwayelu, Afrough et al. 2020).

Studies have been conducted throughout West Africa to establish the presence of CCHF within livestock populations and recently included findings of potential disease endemicity in Senegal (Wilson, LeGuenno et al. 1990, Mangombi, Roqueplo et al. 2020). CCHF outbreaks have been a recurrent observation throughout Mauritania and Senegal (Mangombi, Roqueplo et al. 2020). A late 1980s serological study observed CCHFV seroprevalence of 10.4% in sheep with

antibody prevalence increasing with older age (Wilson, LeGuenno et al. 1990). The need for updated CCHFV epidemiological data in Senegal lead to a seroprevalence study in domesticated animals (Mangombi, Roqueplo et al. 2020). Mangombi, Roqueplo et al. (2020) observed CCHFV seroprevalences of 22.1% (30/136) in sheep and 6.9% (2/29) in goats. The study suggests the presence of high seropositivity in animals indicates the circulation of CCHFV within Senegal and the need to investigate correlation between occurrence of animal infection and human infection within endemic areas (Mangombi, Roqueplo et al. 2020). As The Gambia is a smaller West African country surrounded by Senegal, this study aimed to investigate if CCHF is circulating in The Gambia as well. A collaborative agreement with the West Africa Livestock Innovation Centre (WALIC) implemented a cross-sectional sampling of indigenous goats and sheep within the five regions of The Gambia (Figure 13). Sampling included locations with high human-livestock interaction to assess the potential risk of zoonotic transmission. Serological surveillance in animals provides critical data in detecting the circulation of CCHF in suspected areas. Focusing on species with exposure risk factors and increased human interaction contributes to a better understanding of the epidemiological relationship between vectors and hosts.

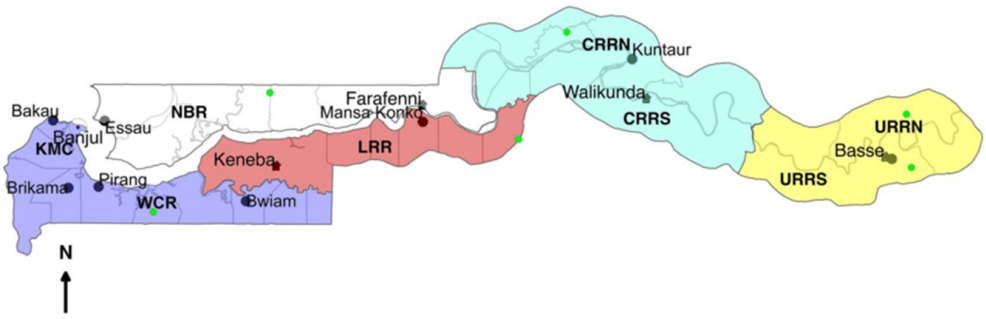

Figure 13. Regional map of The Gambia

4.2 Materials and methods

Study sites

Sampling sites within the Sudano-Guinean zone (Abuko and Brikama) received 900 to 1200 mm of rainfall with maximum daily temperatures range of 26 to 32°C. Vegetation in certain portions of the zone is savannah-woodland or woodland. While areas of the zone around the coast is characterized by humid tropical forest vegetation (Faburay, Jongejan et al. 2008).

Sampling sites within the Western Sudano-Sahelian zone (Keneba, Soma, and Farafenni) average 800 mm of rainfall. Maximum daily temperatures range 28 to 38 °C. Vegetation in this zone is comprised of degraded savannah woodland interspersed with natural grasslands, trees, and farmland. Areas with lowland tree vegetation contain low and high mangroves (Faburay, Jongejan et al. 2008).

The Eastern Sudano-Sahelian zones averages 700 mm of rainfall and maximum daily temperatures range from 30 to 40°C. Sampling sites within this zone (Brikamaba, Sololo, and Basse) possess vegetation that is predominantly savannah interspersed with trees, grasses, and arable farmland. Areas adjacent to the river comprise of riparian woodland interspersed with rice fields (Faburay, Jongejan et al. 2008).

Animals and sample collection

Between April to May 2021, 1,018 serum samples were collected from Djallonké sheep and West African dwarf goats throughout The Gambia in a cross-sectional study within the five regions: Western, Lower River, North Bank, Central River and Upper River. Small ruminants were sampled for serum at eight study sites: 1-Abuko livestock market/abattoir, 2- WALIC Keneba station, 3- Soma livestock market/abattoir, 4- Farafenni livestock market/abattoir, 5- Brikama livestock market, 6- WALIC Sololo station, 7- Basse livestock market, and 8- Brikamaba

Saturday's weekly market (Figure 14). Georeferenced coordinates were taken for each sampling site except for the Brikama livestock market and mapped using ArcGIS. Sampling included animals under and over 6 months of age and of both sexes. The samples were inactivated and shipped to the Foreign Animal Disease Diagnostic Laboratory at Plum Island Animal Disease Center for testing.

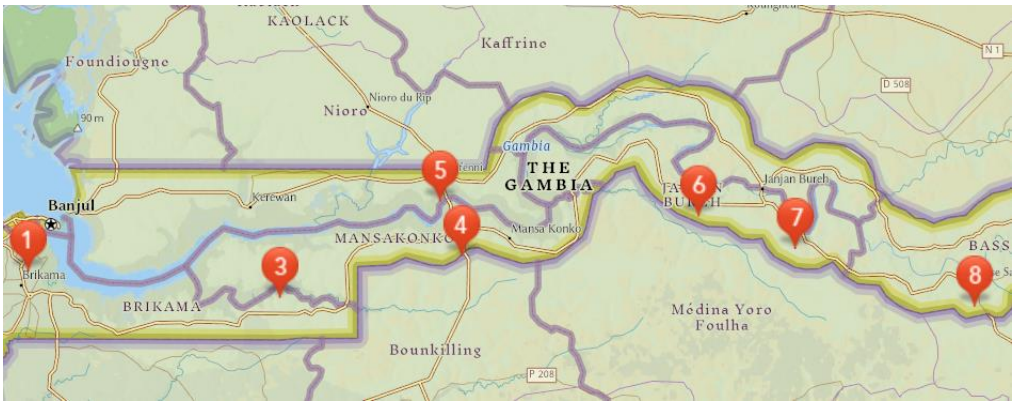

Figure 14. Geographical locations of the sampling sites as depicted in ArcGIS. Georeferenced coordinates were not provided for Brikama livestock market.

Dual antigen enzyme-linked immunosorbent assay (DA ELISA)

The small ruminant samples were tested in duplicate for the presence of antibodies against CCHFV using a validated CCHF double antigen multi-species ELISA kit (ID.Vet, France). The protocol was conducted according to the manufacturer and described previously in chapter 3. Absorbance for each sample was recorded at an optical density (OD) setting of 450 nm. Sample to positive ratio percentage (S/P%) was calculated using the equation as provided by the manufacturer:

$$S/P\,\% = \frac{OD_{SAMPLE}}{OD_{PC}} \times 100$$

Samples that presented with a S/P % ≤ 30% were considered negative, and those presenting with a S/P % ≥ 30% were considered positive.

Tests were validated using the manufacturer's recommendation. Briefly, if the mean OD_{450} of the positive control O.D. (OD_{PC}) is greater than 0.350 and the ratio of the mean values of the positive and negative controls (OD_{PC} and OD_{NC}) is greater than 3, the test is valid:

$$\frac{OD_{PC}}{OD_{NC}} > 3$$

Data Analysis

Data was analyzed using GraphPad Prism and EpiInfo. Comparison for statistical significance in seropositive samples was conducted: (i) between species within a region using Wilcoxon two-sample test (ii) among regions with goats and sheep combined using Kruskal-Wallis one-way analysis of variance and (iii) by age and sex using chi-square test.

4.3 Results

Out of the 1,018 small ruminant samples tested for the presence of CCHFV antibodies, 138 samples were positive. Providing an overall CCHFV seroprevalence of 13.6% (138/1018) (Figure 15) for goats and sheep combined. Seropositivity was about two times higher in sheep at 18.8% (89/474) compared to goats at 9.0% (49/544) and this was shown to be a statistically significant result ($p < 0.0001$) in Figure 16.

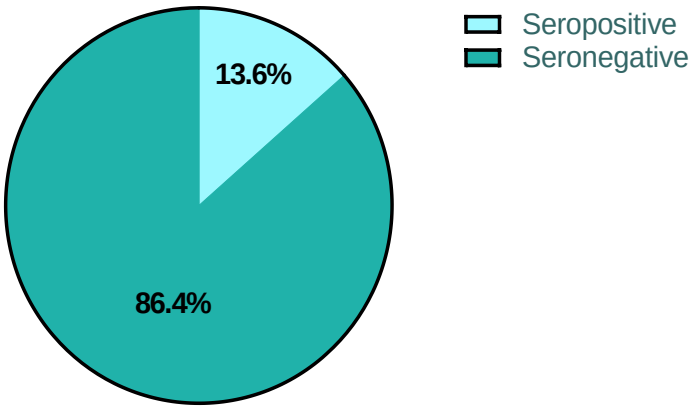

Figure 15. Percentage of seropositive and seronegative animals based on CCHF DA ELISA. n = 1,018

Figure 16. Number of animals sampled versus seropositive based on species

There were two age groups sampled: (i) younger than six months old and (ii) older than six months old. All 138 seropositive animals fell into the above six months old age group. Sampling of younger animals was limited as this age group only included 4.6% (47/1018) of animals and no CCHFV seropositivity was detected in this age group. Sixty-four-point five percent (89/138) seropositive animals were female (Figure 17). There was no difference in CCHF seroprevalence between females 13.5% (89/658) and males 13.6% (49/360).

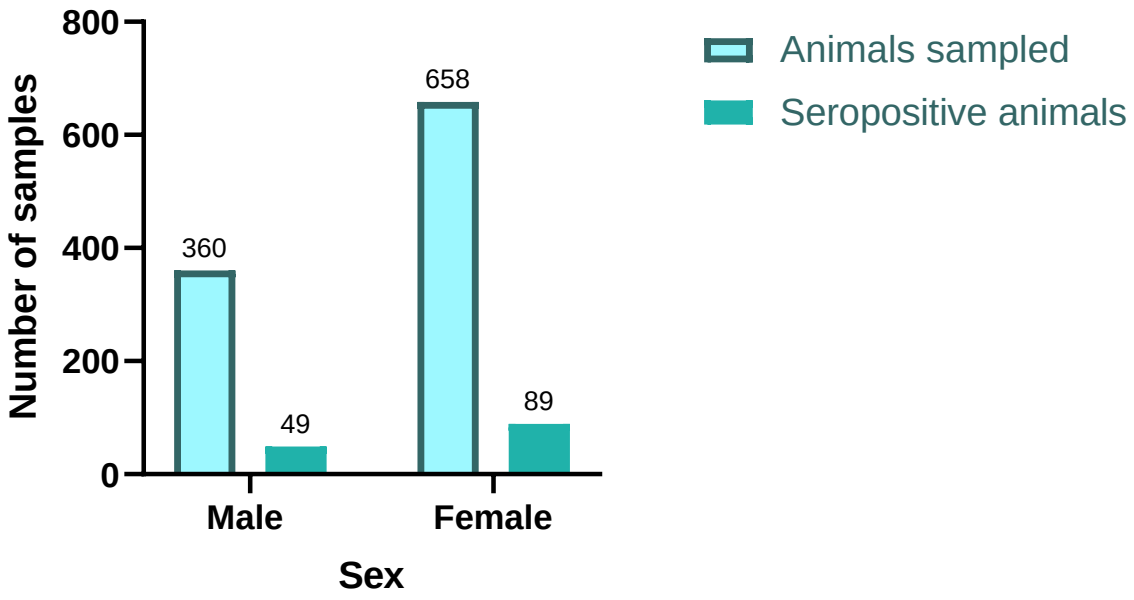

Figure 17. Number of animals sampled versus seropositive based on sex

CCHFV seroprevalence was highest in the Eastern Sudano Sahelian zone for goats 14.8% (22/149), sheep 25.3 % (38/150) and combined goats and sheep 20.1% (60/299) of the three agro-ecological zones in The Gambia. Table 2 provides the percentage of total livestock and the percentage of seropositive animals within each zone.

Table 2. Proportions of total small ruminant populations in the three agro-ecological zones (AEZ) of The Gambia and overall seroprevalence

Agro-ecological zone	% of total livestock			% of seropositive animals (total no. sampled)		
	Goats	Sheep	Total	Goats	Sheep	Combined goats + sheep
Western Sudano Sahelian	46.5	34.0	40.7	4.0 (253)	8.1 (161)	5.6 (414)
Sudano Guinean	26.1	34.4	30.0	12.0 (142)	23.3 (163)	18.0 (305)
Eastern Sudano Sahelian	27.4	31.6	29.3	14.8 (149)	25.3 (150)	20.1 (299)

Regional CCHFV seroprevalence was found to be highest in the Central River region for all three categories (Figure 18). Central River region encompasses two of the sampling sites: Brikamaba Luomo and Sololo WALIC station. CCHFV seropositivity within the CRR was observed as: goats 17.1% (12/70), sheep 25.9% (30/116) and all animals combined 22.6% (42/186). The Western and Upper River regions also exhibited high seropositivity. Table 3 compares the percentage of total livestock to the percentage of seropositive animals for each region with statistical significance being shown ($p < 0.0001$) in all regions except for the North Bank region.

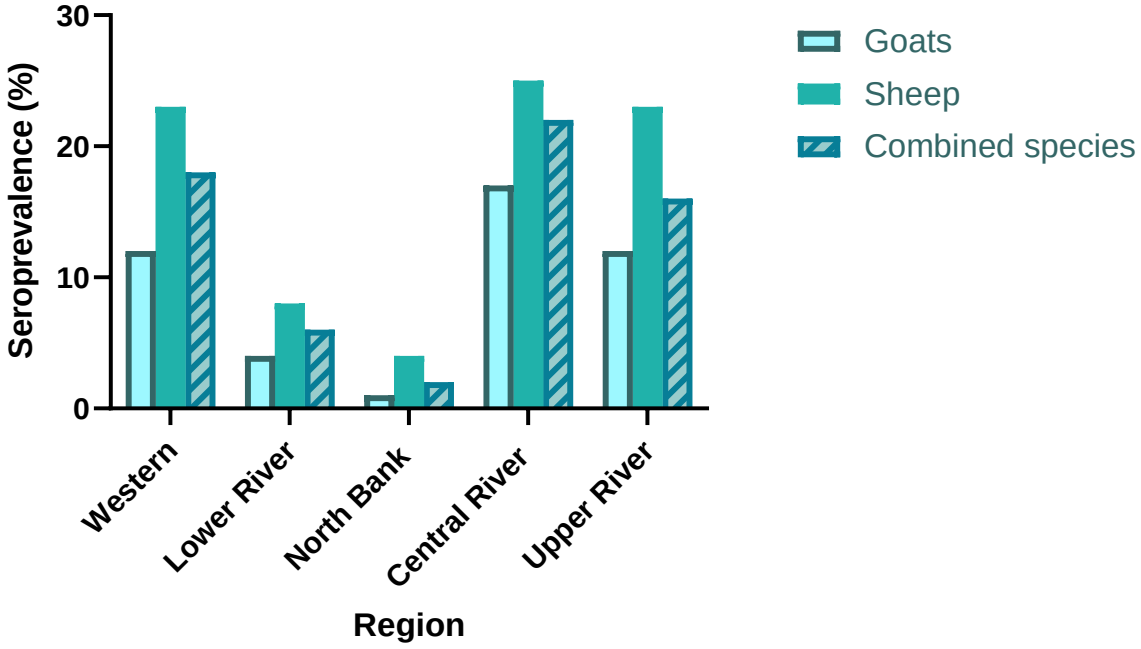

Figure 18. Regional distribution of CCHF seroprevalence in goats and sheep

Table 3. Proportions of total small ruminant populations in the five regions of The Gambia and overall seroprevalence

		% of total livestock			% of seropositive animals (total no. sampled)			
Region	No. of sites	Goats	Sheep	Total	Goats	Sheep	Combined goats + sheep	Probability
Western	2	26.10	34.29	29.96	11.9 (142)	23.3 (163)	18.0 (305)	<0.0001
Lower River	2	36.21	29.54	33.10	4.6 (197)	8.6 (140)	6.2 (337)	<0.0001
North Bank	1	10.29	4.43	7.56	1.8 (56)	4.8 (21)	2.6 (77)	NA
Central River	2	12.87	24.47	18.27	17.1 (70)	25.9 (116)	22.6 (186)	<0.0001
Upper River	1	14.52	7.17	11.10	12.7 (79)	23.5 (34)	15.9(113)	<0.0001

Table 4 provides a breakdown of seroprevalence for each of the eight sampling locations. Sololo WALIC station presented with the highest seroprevalences for goats 31.3% (10/32), sheep 38.1% (24/63) and animals combined 35.8% (34/95) (Figure 19). High CCHFV seropositivity was additionally seen within the Abuko and Basse livestock markets. The remaining five sampling sites all had lower seropositivity in goats, sheep and both species combined.

Table 4. Seroprevalence of goats and sheep for each of the sampling locations and the site number to determine the locale on map

			Seroprevalence (%)		
Region	Site	Site No.	Goats	Sheep	Combined goats + sheep
Western	Abuko livestock market/abattoir	1	19.6 (51)	28.7 (129)	26.1 (180)
	Brikama livestock market	5	7.7 (91)	2.9 (34)	6.4 (125)
Lower River	Keneba WALIC station	2	4.6 (152)	8.6 (129)	6.4 (281)
	Soma livestock market	3	4.4 (45)	9.1 (11)	5.4 (56)
North Bank	Farafenni livestock market	4	1.8 (56)	4.8 (21)	2.6 (77)
Central River	Brikamaba Luomo	8	5.3 (38)	11.3 (53)	8.8 (91)
	Sololo WALIC station	6	31.3 (32)	38.1 (63)	35.8 (95)
Upper River	Basse livestock market	7	12.7 (79)	23.5 (34)	15.9 (113)

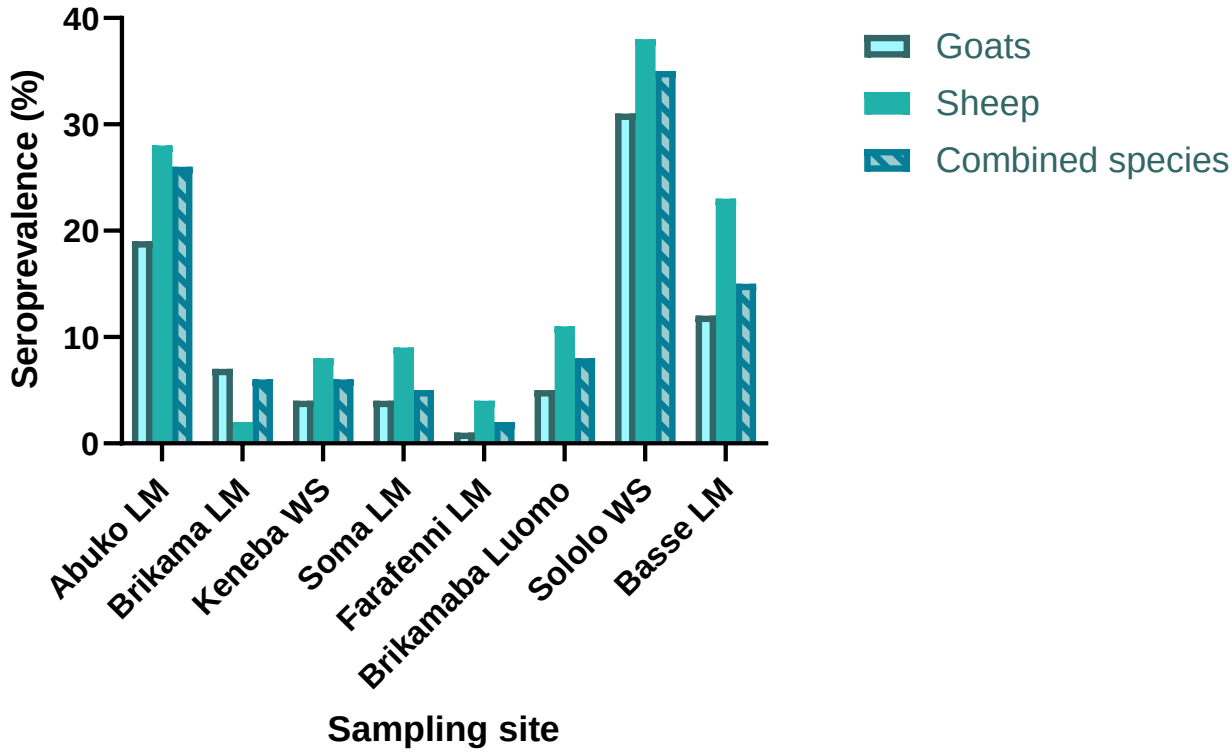

Figure 19. Distribution of CCHF seroprevalence in goats and sheep at 8 sampling sites

4.4 Discussion

To the best of our knowledge this is the first CCHFV seroprevalence study to be conducted in The Gambia, although studies on seroprevalence in humans and animals have been conducted in neighboring Senegal that share ecological similarity with The Gambia (Mangombi, Roqueplo et al. (2020), Wilson, LeGuenno et al. (1990)). The overall CCHFV seroprevalence of 13.6% (138/1018) and the individual CCHFV seroprevalences for goats 9.0% (49/544) and sheep 18.8% (89/474) are consistent with CCHFV seroprevalences that have been shown in small ruminant studies conducted in other countries within West Africa (Mangombi, Roqueplo et al. 2020, Schulz, Barry et al. 2021). Using the same CCHF DA ELISA kit by ID.Vet (France), seroprevalences of 6.9% (2/29) and 22.1% (30/136) were observed in goats and sheep in Northwestern Senegal

(Mangombi, Roqueplo et al. 2020). Similar findings have been observed outside of Africa using an indirect ELISA in Iraq, where the study obtained an overall CCHFV seroprevalence of 14%, with individual seroprevalences at 6.25% and 19.16% in goats and sheep (Altaliby 2021).

The difference in seroprevalence between species was shown to be statistically significant ($p < 0.0001$). Sheep CCHFV seroprevalence was nearly two times higher than that in goats and may be associated with longer term exposure to ticks, although this should be defined in future studies. Wealthier households that own over ten cattle were observed to possess higher numbers of goats and sheep for income, ceremonial/dowry, and lastly meat consumption (Ejlertsen, Poole et al. 2013). Sheep tend to be of more importance ceremonially in The Gambia compared to goats and may be kept longer to older age resulting in higher risk of exposure to tick vectors and other risk factors in sheep. CCHFV antibody prevalence increases with age, thus sheep kept for longer periods of time have greater risk of exposure to CCHFV positive ticks. High seroprevalence of CCHFV in sheep presents increased risk of exposure to CCHFV infection among workers at slaughterhouses and abattoirs.

Previous studies conducted throughout West Africa present mixed results as to whether CCHFV seroprevalences are impacted by age or sex. For instance, a study in livestock in Mauritania found age to be statistically significant for CCHFV seroprevalences and results showed seroprevalences increased with age. Sex was not found to be statistical significant (Schulz, Barry et al. 2021). While this study was limited in the number of young animals sampled, age was a statistically significant factor, X^2 (1, N=1,018) = 6.641, p = 0.0100; apparently older animals tend to carry more ticks, which may influence the CCHFV seroprevalence in the different age categories among species. Sex did not appear to be a contributing factor to exposure and was not shown to

be a statistically significant in this study X^2 (1, N=1,018) = 0.001099, p = 0.9736. This may be due to the large disproportionate sampling of females.

Increased CCHFV antibody seroprevalence within the Eastern Sudano Sahelian zone may be explained by the ideal climatic conditions of the area. A 1990 study in Senegal by Wilson et al., found that there was a negative relationship between regions with higher precipitation and increased CCHF transmission activity. Sheep CCHFV seropositivity decreased from 75.0% in regions with lower precipitation to 0% in those with high precipitation (Wilson, LeGuenno et al. 1990). A more recent study in Tunisia by Zouaghi, Bouattour et al. (2021) found a similar result in that there was a positive correlation between bioclimatic zones and CCHFV seropositivity. The study found bioclimatic zones to be a potential risk factor to consider as climate changes can affect tick distribution and in turn how the virus circulates (Zouaghi, Bouattour et al. 2021). The Eastern Sudano Sahelian is a predominantly semi-arid region and may be more ideal for the primary CCHFV vector, *Hyalomma* ticks, to be maintained in the environment. *Hyalomma* ticks are known to be active hunters and the savannah vegetation interspersed with grasses provides active coverage. Tick dispersal throughout The Gambia would need to be studied to identify which areas possess high tick prevalence. Ninety-six percent and 99% of goat and sheep livestock owners allow their animals to graze freely during the dry season (November to June) (Ejlertsen, Poole et al. 2013) and any increased risk to CCHFV positive tick exposure to these animals may spillover into risk of exposure to farmers or abattoir workers.

Within the Eastern Sudano Sahelian zone is the Central River region, which contained the highest CCHFV seroprevalence among the five regions and differences between the combined species regionally was shown to be statistically significant ($p<0.0001$) except in the North Bank region due to limitation of samples. This finding is primarily due to Sololo WALIC station being

the site with the highest CCHFV antibody seroprevalence. As WALIC stations focus on livestock breeding and genetic improvement, the animal movement to and from the facility may play a role in the high CCHFV antibody seroprevalence shown. The research on livestock breeding may be another factor as animals in these programs tend to be kept for longer periods of time compared to an animal used for farming purposes. Brikamaba Luomo is located within the Central River region in the Eastern Sudano-Sahelian zone but showed CCHFV seroprevalences that were more in line with that of the sites within the Lower River and North Bank regions located in the Western Sudano-Sahelian zone. Livestock movement and trade may be an influencing factor in exposing animals to ticks and consequently impacts on the seroprevalence of infection.

4.5 Conclusions

The study aimed to identify if CCHF infections were prevalent in the small ruminant populations of The Gambia. These findings demonstrate that CCHFV exposure risks are present, and CCHF may potentially be endemic within the country. This data can be used to establish surveillance based on the sites of greatest risk and to further study the epidemiology of CCHFV in The Gambia.

Chapter 5 - Crimean Congo hemorrhagic fever seroprevalence in cattle of The Gambia

5.1 Introduction

Crimean Congo hemorrhagic fever (CCHF), a severe form of hemorrhagic fever, is endemic in Africa, Asia, Middle East and Eastern Europe (Appannanavar and Mishra 2011). Knowledge is still lacking on the potentiality of a country to be endemic for the disease in many of these regions and creates challenges in the implementation of CCHF control measures needed for the CCHF suspect country and its neighbors (Appannanavar and Mishra 2011). Many domestic and wild animals (including cattle, goats, sheep, reptiles, and ostriches) act as asymptomatic hosts of CCHFV that are critical to the transmission cycle of the primary vector, *Hyalomma* ticks (Nasirian 2019, Temur, Kuhn et al. 2021). Due to domestic and wild animals being asymptomatic, the use of serological studies to identify animals exposed to CCHFV has become a primary surveillance method in detecting the presence of natural transmission in CCHF suspected areas (Spengler, Estrada-Pena et al. 2016).

Serological surveillance in animals provides critical data in detecting the circulation of CCHFV in suspected areas. Focusing on species with exposure risk factors and increased human interaction creates a better understanding of the epidemiological relationship between vectors and hosts. Previous studies throughout West Africa established cattle are a preferred host of *Hyalomma* ticks and high seroprevalences have been reported in infected animals ranging from 24% to 69% (Mangombi, Roqueplo et al. 2020, Oluwayelu, Afrough et al. 2020, Blanco-Penedo, Obanda et al. 2021, Schulz, Barry et al. 2021). Studies have shown a positive correlation between high CCHF seropositivity in cattle and the number of ticks found on an animal, (Msimang, Weyer et al. 2021, Zouaghi, Bouattour et al. 2021) making cattle suitable indicator animals in serological studies

assessing the risk of human exposure (Hartlaub, Daodu et al. 2021). In 2021, validated CCHF multi-species dual antigen ELISA kits were used to examine cattle sera samples in Uganda. Researchers observed CCHFV seropositivity of 75.0% in cattle and found it was associated with location, increasing age, high tick burden and females (Balinandi, von Bromssen et al. 2021). Although several studies on the seroprevalence and risk of CCHF have been carried out in Senegal, the lack of specific and up to date information on the seroprevalence of infection in host species, especially cattle, an important target species in The Gambia, represents a significant gap in our knowledge regarding the risk and epidemiology of the disease in the country. Consequently, a cross-sectional study was carried out to investigate the seroprevalence of CCHF at selected sites among indigenous cattle population (N'Dama cattle) in The Gambia.

5.2 Material and Methods

Study sites

In collaboration with the West Africa Livestock Innovation Centre (WALIC), sampling of N'Dama cattle from 16 herds across 10 villages in The Gambia was carried out (Figure 20). The villages were located in a cluster within the Lower River region. Georeferenced coordinates were recorded for mapping using ArcGIS. The Lower River Region covers 1,618 km^2 of area and a total population of 25, 268 cattle (i.e. 23,640 N'Dama and 1,628 N'Dama draught cattle) based on the 2016 livestock census (Loum 2019, Mendy, Azinwie et al. 2020). The region is characterized by extended periods of dryness, longer growing season, and an average rainfall of 600 – 900 mm (Mendy, Azinwie et al. 2020).

Animals and sample collection

N'Dama cattle were the primary cattle breed sampled. They are the predominant breed in Gambia and are more resistant to heat, drought, feed scarcity, parasites, and vector borne diseases compared to other breeds (Loum 2019).

During the early dry season (February 2021), 399 N'Dama cattle were sampled from local villages. Herd sizes ranged from 45 to 283 animals. The number of herds sampled per village ranged from 1 to 3 with sample size ranging from 30 to 65 cattle (Table 5). Both females and males were sampled, and three different age groups were included: calves, young adults (between 2 to 4 years old) and adults (more than 4 years old). Samples were inactivated and sent to the Foreign Animal Disease Diagnostic Laboratory at Plum Island Animal Disease Center for testing.

Table 5. Sampling frame showing the number of cattle herds, herd size and number of cattle sampled per herd

Village	Herd number	Herd size	n sampled
Wudeba	1	73	50
Bateling	2	45	40
Dumbuto	3 4 5	70 35 70	20 20 25
Sankandi	6 7	35 75	25 20
Niorro Jataba	8 9	78 40	24 25
Bajana	10	65	30
Jiffarong	11 12	90 60	15 15
Jali	13	70	30
Kuli Kunda	14 15	120 163	15 15
Tankular	16	150	30
Total		1239	399

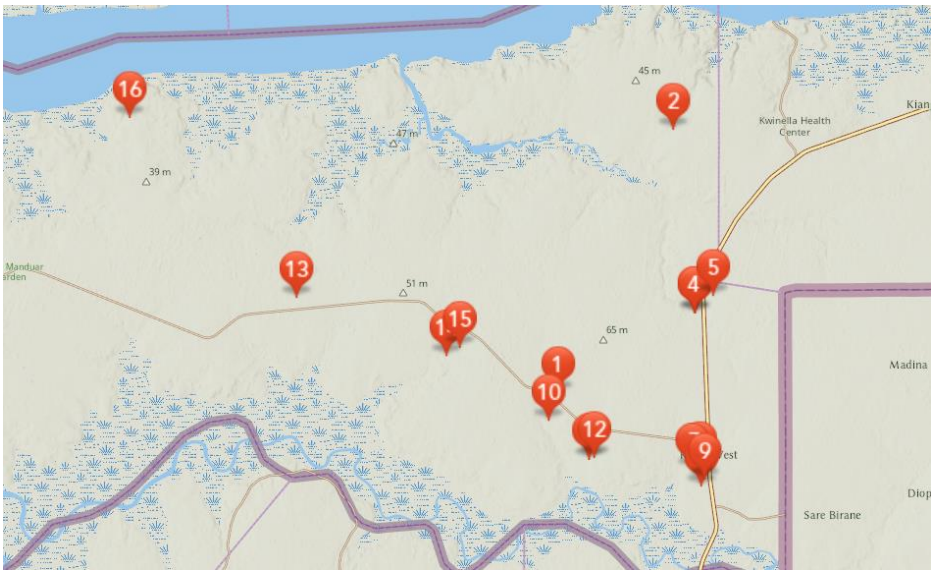

Figure 20. Mapping of sampled villages based on location of each individual herd

Dual Antigen enzyme linked immunosorbent assay (DA ELISA)

Cattle serum samples were tested in duplicate for the presence of CCHFV antibodies using a validated multi-species DA ELISA kit (ID.Vet, France). The protocol was followed as instructed by the manufacturer and detailed in chapter 3. Optical density (OD) was set at 450 nm and absorbance for each sample was recorded. Sample to positive ratio percentage (S/P%) was calculated using the equation as provided by the manufacturer:

$$S/P\,\% = \frac{OD_{SAMPLE}}{OD_{PC}} \times 100$$

Samples that presented with a S/P % ≤ 30% were considered negative, and those presenting with a S/P % ≥ 30% were considered positive.

Test validation was completed using the manufacturer's recommendation. If the mean OD_{450} of the positive control O.D. (OD_{PC}) is greater than 0.350 and the ratio of the mean values of the positive and negative controls (OD_{PC} and OD_{NC}) is greater than 3, the test is valid:

$$\frac{OD_{PC}}{OD_{NC}} > 3$$

Data Analysis

Data was analyzed using GraphPad Prism and EpiInfo. Comparison for statistical significance in seropositive samples was conducted: (i) between villages using Kruskal-Wallis one-way analysis and (ii) by age and sex using chi-square test.

5.3 Results

Of the 399 cattle sampled, 239 were positive for antibodies against CCHFV. Providing an overall CCHFV seroprevalence of 59.9% (239/399) as shown in Figure 21.

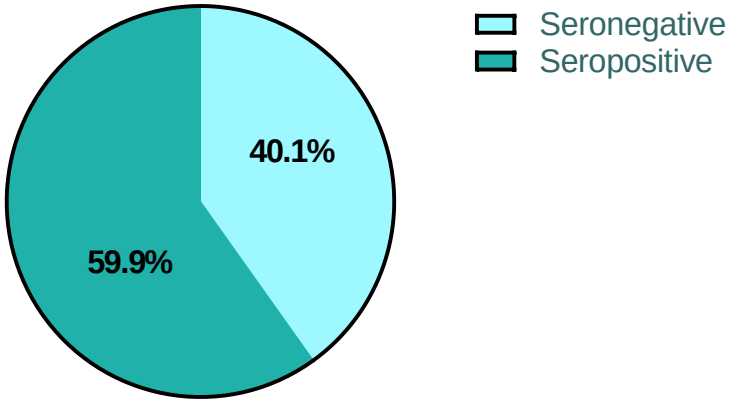

Figure 21. Distribution of seropositive and seronegative samples from CCHF DA ELISA testing. n = 399

CCHFV seropositivity was higher in females 64.2% (194/302) compared to males 46.4% (45/97). A difference that was not shown to be statistically significant X^2 (1, N=399) = 2.596, p = 0.1071. Figure 22 compares the sampling size of females and males to the number of seropositive cattle in each category. The number of females sampled was around three times higher than the sampling of males.

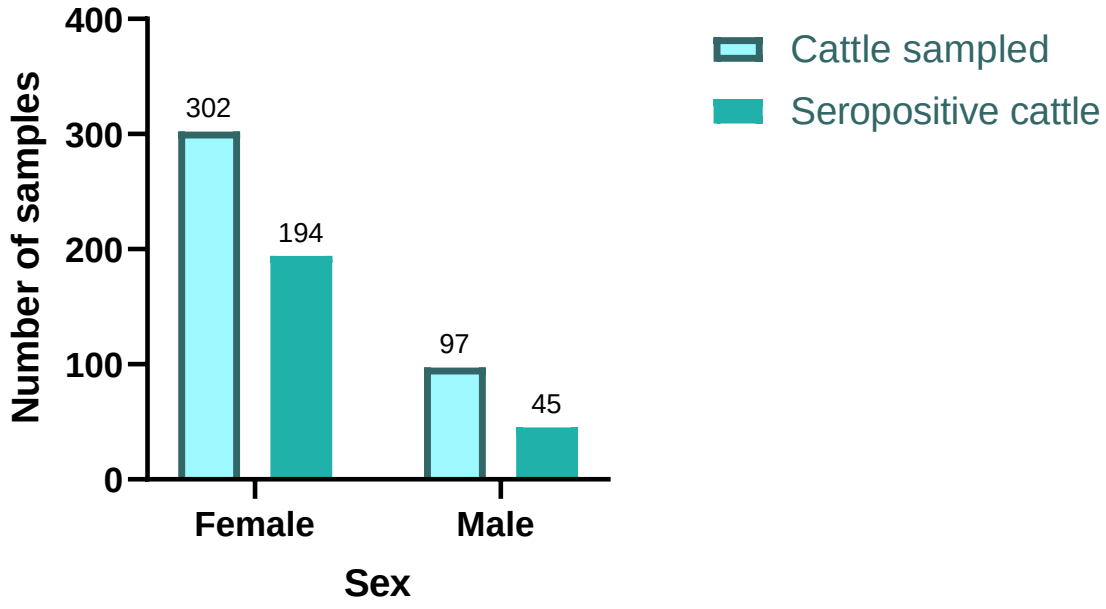

Figure 22. Number of cattle sampled versus seropositive based on sex

Cattle were separated by age group into calves, young adults (between two to four years old) and adults (more than four years old). CCHFV seroprevalence observed in each age category was: calves 61.0% (17/28), young adults 55.8% (92/165), and adults 63.1% (130/206). Age group differences were not statistically significant X^2 (2, N=399) = 0.5222, p = 0.7702 when analyzed. Figure 23 compares sampling size to number of seropositive animals based on age group.

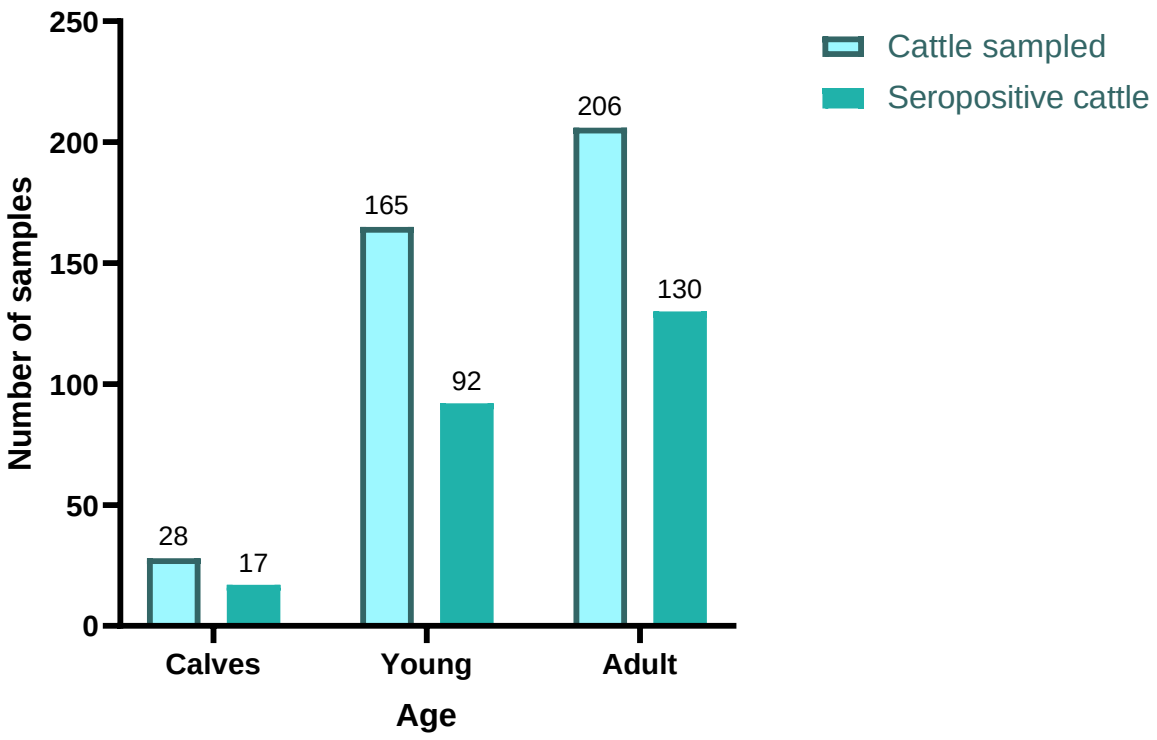

Figure 23. Number of cattle sampled versus seropositive within the three age groups

The highest CCHFV seroprevalence in cattle was observed in Wudeba village at 84.0% (42/50) (Figure 24). Cattle CCHFV seropositivity fluctuated between villages and remained high throughout, ranging from 30.0% to 84.0%. The differences in seroprevalence between villages was shown to be statistically significant ($p < 0.0001$).

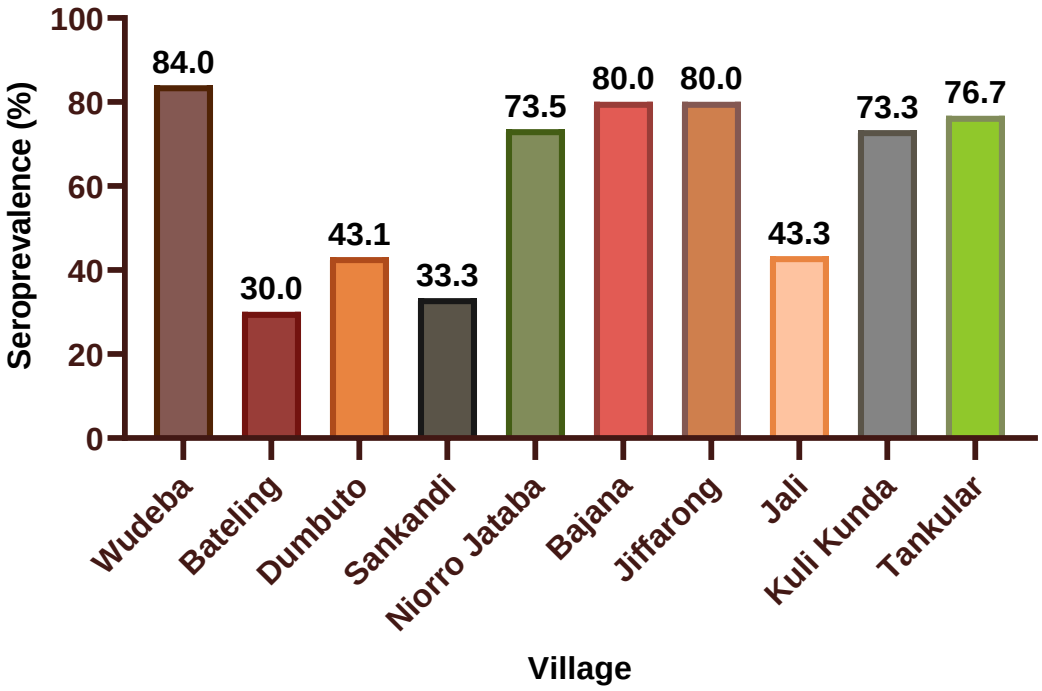

Figure 24. Overall seroprevalence of CCHF at each village in the study

5.4 Discussion

When comparing CCHFV seroprevalence among livestock species, cattle tend to present the highest seropositivity. A previous study conducted in Mauritania found a seroprevalence of 69% in cattle using an indirect ELISA and the authors reported higher seropositivity with increasing age (Schulz, Barry et al. 2021). Increased seropositivity in cattle may be attributed to longevity of animals and long-term exposure to infected tick bites. Tick infestation is normally high on cattle and studies have suggested that the number of ticks attached may increase as cattle age increases (Balinandi, von Bromssen et al. (2021), Msimang, Weyer et al. (2021)).

Older cattle may have increased risk of direct contact exposure to infected ticks due to their longevity compared to younger cattle. The villages are located within the Lower River region of the Western Sudano Sahelian zone; an area characterized by degraded savannah woodlands with

natural grasses, trees, and farmland (Faburay, Jongejan et al. (2008)). The early dry season may be more suitable for *Hyalomma* ticks to thrive as moisture is lowered and vegetation found near these villages provides suitable habitat for ticks and increased risk of tick infestation. Farmers tether their cattle to pegs overnight and bring herds to graze in communal lands during the day as their main feed source (Loum 2019). Calves may have exhibited relatively high seropositivity to CCHF antibodies due to horizontal transmission as they typically get infested with ticks early before weaning. It is unclear if transfer of antibodies through the colostrum contributes to the prevalence. A study by Levieux (1984), as cited by Gonzalez, Camicas et al. (1998), found lambs received antibodies through feeding on the colostrum of their CCHF infected mothers.

The findings showed a higher CCHFV seroprevalence in females than males, although both may have equal exposure to risk factors including the opportunity to be infected by ticks. However, sampling favored female cattle heavily and disproportionate sampling could indicate potential selection bias within the study. Alternatively, females could potentially be housed for longer and in areas of high exposure risks. N'Dama cattle are reared for milk and meat production with breeding females or males being sold to other breeders (Loum 2019). Bulls that are no longer adequate breeding stock may be sent to slaughter, but cows may be kept longer for milk production once breeding capabilities are lost and they have increased risk of exposure to tick transmission.

Overall CCHFV seroprevalences were relatively high in cattle for all villages. The differences in seroprevalences between villages and reasoning as to why Bateling, Sankandi, Dumbuto and Jali are lower ranges of 30% - 43% is uncertain. Majority of the villages are clustered closer in proximity and that should suggest similar levels of seroprevalence and disease risk in geographic locality. The lower seroprevalence villages are located further away from this central cluster and may have lower tick abundance resulting in lower host transmission. The exception to

that are the farms located in Sankandi that are near those in Niorro Jataba. As well as Tankular, which is located furthest out from all other villages and had a CCHFV seroprevalence of 76.7%. It is noteworthy, that slight variations in cattle rearing practices may also influence host exposure to risk factors including the degree of exposure to infected tick bite. To understand the CCHFV seroprevalence differences between villages, additional work would need to include identifying the tick abundance, local vegetation, and cattle rearing practices for the villages sampled.

5.5 Conclusion

This study built upon the work on small ruminants in chapter 4 and further established that CCHF may be endemic in The Gambia. There are mixed views on small ruminants being indicator species when identifying the epidemiological significance of CCHF within new areas. However, cattle appear to be the preferred indicator for assessing the epidemiological significance of CCHF within new areas. Considering that cattle generally carry higher tick infestation and overall, consistently exhibit high seroprevalences, there is validity in that reasoning. Identifying cattle as an indicator species for CCHF will provide insight into the strategy for CCHF surveillance and assessing disease risk in The Gambia, and critical needs to establish protocols to protect public health, particularly abattoir workers and farmers that may handle these animals frequently.

References

(WHO), W. H. O. (2017). "Crimean-Congo Hemorrhagic Fever." from https://www.who.int/health-topics/crimean-congo-haemorrhagic-fever#tab=tab_1.
Abubakar, I. R. (2014). "Abuja city profile." Cities **41**: 81-91.

Akinci, E., H. Bodur and H. Leblebicioglu (2013). "Pathogenesis of Crimean-Congo hemorrhagic fever." Vector Borne Zoonotic Dis **13**(7): 429-437.

Akuffo, R., J. A. 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 Infect Dis **16**: 324.

Altaliby, M. A. S., S. A. Esmaeel, Kh. J. Hussain (2021). "Seroprevalence of Crimean-Congo Haemorrhagic Fever in sheep and goats in Iraq." Bulgarian Journal of Veterinary Medicine.
Appannanavar, S. B. and B. Mishra (2011). "An update on crimean congo hemorrhagic Fever." J Glob Infect Dis **3**(3): 285-292.

Aradaib, I. E., B. R. Erickson, M. E. Mustafa, M. L. Khristova, N. S. Saeed, R. M. Elageb and S. T. Nichol (2010). "Nosocomial Outbreak of Crimean-Congo Hemorrhagic Fever, Sudan." Emerging Infectious Diseases **16**(5): 837-839.

Balinandi, S., C. von Bromssen, 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." J Virol Methods **290**: 114075.

Bente, D. A., N. L. Forrester, D. M. Watts, A. J. McAuley, C. A. Whitehouse and M. Bray (2013). "Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity." Antiviral Res **100**(1): 159-189.

Blanco-Penedo, I., V. Obanda, E. Kingori, B. Agwanda, C. Ahlm and O. W. Lwande (2021). "Seroepidemiology of Crimean-Congo Hemorrhagic Fever Virus (CCHFV) in Cattle across Three Livestock Pastoral Regions in Kenya." Dairy **2**(3): 425-434.

Bukbuk, D. N., S. D. Dowall, K. Lewandowski, A. Bosworth, S. S. Baba, A. Varghese, R. J. Watson, A. Bell, B. Atkinson and R. Hewson (2016). "Serological and Virological Evidence of Crimean-Congo Haemorrhagic Fever Virus Circulation in the Human Population of Borno State, Northeastern Nigeria." PLoS Negl Trop Dis **10**(12): e0005126.

Burt F.J., P. J. T., Swanepoel R. (2007). Crimean-Congo Hemorrhagic Fever in South Africa. Crimean-Congo Hemorrhagic Fever. W. C. A. Ergonul O., Springer, Dordrecht.

Burt, F. J., J. T. Paweska, B. Ashkettle and R. Swanepoel (2009). "Genetic relationship in southern African Crimean-Congo haemorrhagic fever virus isolates: evidence for occurrence of reassortment." Epidemiol Infect **137**(9): 1302-1308.

Burt, F. J. and R. Swanepoel (2005). "Molecular epidemiology of African and Asian Crimean-Congo haemorrhagic fever isolates." Epidemiol Infect **133**(4): 659-666.

Causey, O. R., G. E. Kemp, M. H. Madbouly and T. S. David-West (1970). "Congo virus from domestic livestock, African hedgehog, and arthropods in Nigeria." Am J Trop Med Hyg **19**(5): 846-850.

Causey, O. R., G. E. Kemp, M. H. Madbouly and V. H. Lee (1969). "Arbovirus surveillance in Nigeria, 1964-1967." Bull Soc Pathol Exot Filiales **62**(2): 249-253.

Chen, J. P. and T. M. Cosgriff (2000). "Hemorrhagic fever virus-induced changes in hemostasis and vascular biology." Blood Coagul Fibrinolysis **11**(5): 461-483.

Chisholm, K., E. Dueger, N. T. Fahmy, H. A. Samaha, A. Zayed, M. Abdel-Dayem and J. T. Villinski (2012). "Crimean-congo hemorrhagic fever virus in ticks from imported livestock, Egypt." Emerging Infectious Diseases **18**(1): 181-182.

David-West, T. S., A. R. Cooke and A. S. David-West (1974). "Seroepidemiology of Congo virus (related to the virus of Crimean haemorrhagic fever) in Nigeria." Bull World Health Organ **51**(5): 543-546.

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." J Virol **80**(17): 8834-8842.

Dunster, L., M. Dunster, V. Ofula, D. Beti, F. Kazooba-Voskamp, F. Burt, R. Swanepoel and K. M. DeCock (2002). "First documentation of human Crimean-Congo hemorrhagic fever, Kenya." Emerg Infect Dis **8**(9): 1005-1006.

Duygu, F., T. Sari, T. Kaya, O. Tavsan and M. Naci (2018). "The Relationship between Crimean-Congo Hemorrhagic Fever and Climate: Does Climate Affect the Number of Patients?" Acta Clin Croat **57**(3): 443-448.

Eguaroje, E., A. A, J. Ogbole, O. S, A. Ali, I. Kolawole, K. Muibi, O. D, O. Popoola, S. Samson, J. Adewoyin, J. I.A, R. Badru, A. Atijosan and O. Ajileye (2015). "Flood Vulnerability Assessment of Ibadan City, Oyo State, Nigeria." **volume 5**: 149-159.

Ejlertsen, M., J. Poole and K. Marshall (2013). "Traditional breeding objectives and practices of goat, sheep and cattle smallholders in The Gambia and implications in relation to the design of breeding interventions." Trop Anim Health Prod **45**(1): 219-229.

el-Azazy, O. M. and E. M. Scrimgeour (1997). "Crimean-Congo haemorrhagic fever virus infection in the western province of Saudi Arabia." Trans R Soc Trop Med Hyg **91**(3): 275-278.

Emmerich, P., T. Avsic-Zupanc, S. Chinikar, A. Saksida, C. Thome-Bolduan, A. Parczany-Hartmann, A. G. Langroudi, M. Moradi, S. Ahmeti, S. Gunther and J. Schmidt-Chanasit (2010). "Early serodiagnosis of acute human Crimean-Congo hemorrhagic fever virus infections by novel capture assays." J Clin Virol **48**(4): 294-295.

Emmerich, P., A. Mika, R. von Possel, A. Rackow, Y. Liu, H. Schmitz, S. Gunther, K. Sherifi, B. Halili, X. Jakupi, L. Berisha, S. Ahmeti and C. Deschermeier (2018). "Sensitive and specific detection of Crimean-Congo Hemorrhagic Fever Virus (CCHFV)-Specific IgM and IgG antibodies in human sera using recombinant CCHFV nucleoprotein as antigen in mu-capture and IgG immune complex (IC) ELISA tests." PLoS Negl Trop Dis **12**(3): e0006366.

Estrada-Pena, A., F. Ruiz-Fons, P. Acevedo, C. Gortazar and J. de la Fuente (2013). "Factors driving the circulation and possible expansion of Crimean-Congo haemorrhagic fever virus in the western Palearctic." J Appl Microbiol **114**(1): 278-286.

Evans, T. S., T. W. Myat, N. S. Hom, K. M. Ricks, M. T. Maw, Z. M. Oo, A. T. Toe, N. T. Aung, P. Aung, T. H. Aung, P. Kuehnert, K. Z. Thant, Y. T. Win, W. Z. Thein, N. R. Gardner, R. J. Schoepp, C. K. Johnson and H. M. Thu (2021). "Seroepidemiologic Survey of Crimean-Congo Hemorrhagic Fever Virus in Logging Communities, Myanmar." Emerg Infect Dis **27**(6): 1709-1713.

Faburay, B., F. Jongejan, A. Taoufik, A. Ceesay and D. Geysen (2008). "Genetic diversity of Ehrlichia ruminantium in Amblyomma variegatum ticks and small ruminants in The Gambia determined by restriction fragment profile analysis." Vet Microbiol **126**(1-3): 189-199.

Fernandez-Garcia MD, N. A., Papa A, Donoso-Mantke O, Niedrig M, Zeller H, Tenorio A, Franco L, the ENVID members (2014). "European survey on laboratory preparedness, response and diagnostic capacity for Crimean-Congo haemorrhagic fever, 2012." Eurosurveillance **19**(26).

Fisher-Hoch, S. P., J. B. McCormick, R. Swanepoel, A. Van Middlekoop, S. Harvey and H. G. Kustner (1992). "Risk of human infections with Crimean-Congo hemorrhagic fever virus in a South African rural community." The American journal of tropical medicine and hygiene **47**(3): 337-345.

Gargili, A., A. Estrada-Pena, J. R. Spengler, A. Lukashev, P. A. Nuttall and D. A. Bente (2017). "The role of ticks in the maintenance and transmission of Crimean-Congo hemorrhagic fever virus: A review of published field and laboratory studies." Antiviral Res **144**: 93-119.

Gonzalez, J.-P., J.-L. Camicas, J.-P. Cornet and M. Wilson (1998). "Biological and clinical responses of West African sheep to Crimean-Congo haemorrhagic fever virus experimental infection." Research in virology **149**(6): 445-455.

Hartlaub, J., O. B. Daodu, B. Sadeghi, M. Keller, J. Olopade, D. Oluwayelu and M. H. Groschup (2021). "Cross-Reaction or Co-Infection? Serological Discrimination of Antibodies Directed against Dugbe and Crimean-Congo Hemorrhagic Fever Orthonairovirus in Nigerian Cattle." Viruses **13**(7): 1398.

Hawman, D. W. and H. Feldmann (2018). "Recent advances in understanding Crimean-Congo hemorrhagic fever virus." F1000Res **7**.

Hoogstraal, H. (1979). "The Epidemiology of Tick-Borne Crimean-Congo Hemorrhagic Fever in Asia, Europe and Africa." Journal of Medical Entomology **15**(4): 307-417.

Kouhpayeh, H. (2021). "A Systematic Review of Treatment Strategies Including Future Novel Therapies in Crimean-Congo Hemorrhagic Fever." International Journal of Infection **8**(2).

Levieux, D. (1984). "Transmission de l'immunité passive colostrale: le point des connaissances." Physiologie et pathologie périnatales chez les animaux de farme, INRA Paris: 345-369.
Loum, B. (2019). National Strategy and Action Plan for Animal Genetic Resources in The Gambia. Banjul. **Final Report**.

Lukashev, A. N., A. S. Klimentov, S. E. Smirnova, T. K. Dzagurova, J. F. Drexler and A. P. Gmyl (2016). "Phylogeography of Crimean Congo Hemorrhagic Fever Virus." PLoS One **11**(11): e0166744.

Lwande, O. W., Z. Irura, C. Tigoi, E. Chepkorir, B. Orindi, L. Musila, M. Venter, A. Fischer and R. Sang (2012). "Seroprevalence of Crimean Congo hemorrhagic fever virus in Ijara District, Kenya." Vector Borne Zoonotic Dis **12**(9): 727-732.

Makama, G. A. (2013). "PATRIARCHY AND GENDER INEQUALITY IN NIGERIA: THE WAY FORWARD." European Scientific Journal, ESJ **9**(17).

Maltezou, H. C. and A. Papa (2010). "Crimean-Congo hemorrhagic fever: risk for emergence of new endemic foci in Europe?" Travel Med Infect Dis **8**(3): 139-143.

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 Zoonotic Dis **20**(10): 797-799.

Mendy, J., A. Azinwie and N. Ndip (2020). "Vulnerability to food insecurity and coping strategies of agrarian households in the lower river region of the Gambia: Implication for policy." International Journal of Agricultural Science and Food Technology: 115-126.

Mertens, M., K. Schmidt, A. Ozkul and M. H. Groschup (2013). "The impact of Crimean-Congo hemorrhagic fever virus on public health." Antiviral Res **98**(2): 248-260.

Meurens, F., C. Dunoyer, C. Fourichon, V. Gerdts, N. Haddad, J. Kortekaas, M. Lewandowska, E. Monchatre-Leroy, A. Summerfield, P. J. Wichgers Schreur, W. H. M. van der Poel and J. Zhu (2021). "Animal board invited review: Risks of zoonotic disease emergence at the interface of wildlife and livestock systems." Animal **15**(6): 100241.

Moore, D. L., O. R. Causey, D. E. Carey, S. Reddy, A. R. Cooke, F. M. Akinkugbe, T. S. David-West and G. E. Kemp (1975). "Arthropod-borne viral infections of man in Nigeria, 1964-1970." Ann Trop Med Parasitol **69**(1): 49-64.

Morrill, J. C., A. K. Soliman, I. Z. Imam, B. A. Botros, M. I. Moussa and D. M. Watts (1990). "Serological evidence of Crimean-Congo haemorrhagic fever viral infection among camels imported into Egypt." J Trop Med Hyg **93**(3): 201-204.

Mostafavi, E., B. Pourhossein, S. Esmaeili, F. Bagheri Amiri, S. Khakifirouz, N. Shah-Hosseini and S. M. Tabatabaei (2017). "Seroepidemiology and risk factors of Crimean-Congo Hemorrhagic Fever among butchers and slaughterhouse workers in southeastern Iran." Int J Infect Dis **64**: 85-89.

Mousavi-Jazi, M., H. Karlberg, A. Papa, I. Christova and A. Mirazimi (2012). "Healthy individuals' immune response to the Bulgarian Crimean-Congo hemorrhagic fever virus vaccine." Vaccine **30**(44): 6225-6229.

Msimang, V., J. Weyer, C. le Roux, A. Kemp, F. J. Burt, S. Tempia, A. Grobbelaar, N. Moolla, M. K. Rostal, W. Bagge, C. Cordel, W. B. Karesh, J. T. Paweska and P. N. Thompson (2021). "Risk factors associated with exposure to Crimean-Congo haemorrhagic fever virus in animal workers and cattle, and molecular detection in ticks, South Africa." PLoS Negl Trop Dis **15**(5): e0009384.

Muianga, A. F., R. Watson, A. Varghese, I. S. Chongo, S. Ali, V. Monteiro, F. Inalda, I. Chelene, V. Antonio, R. Hewson and E. S. Gudo (2017). "First serological evidence of Crimean-Congo haemorrhagic fever in febrile patients in Mozambique." Int J Infect Dis **62**: 119-123.
Nasirian, H. (2019). "Crimean-Congo hemorrhagic fever (CCHF) seroprevalence: A systematic review and meta-analysis." Acta Trop **196**: 102-120.

Nikolich-Žugich, J. (2014). "Aging of the T cell compartment in mice and humans: from no naive expectations to foggy memories." J Immunol **193**(6): 2622-2629.

Oklu M., S. I., Altuntas C., Dane S. (2020). "Recombination Analysis of S-Segment Genome of Crimean-Congo hemorrhagic fever virus in turkey." Journal of Research in Medical and Dental Science **8**(4): 141-145.

Okorie, T. G. (1991). "Comparative studies on the vector capacity of the different stages of Amblyomma variegatum Fabricius and Hyalomma rufipes Koch for Congo virus, after intracoelomic inoculation." Vet Parasitol **38**(2-3): 215-223.

Oluwayelu, D., B. Afrough, A. Adebiyi, A. Varghese, P. Eun-Sil, S. Fukushi, T. Yoshikawa, M. Saijo, E. Neumann, S. Morikawa, R. Hewson and O. Tomori (2020). "Prevalence of Antibodies to Crimean-Congo Hemorrhagic Fever Virus in Ruminants, Nigeria, 2015." Emerg Infect Dis **26**(4): 744-747.

Oluwole, O. and N. Daful (2014). "Characterization of Jos City Road Network, Nigeria." Indonesian Journal of Geography **46**: 177.

Pandya, J. and D. S. Rajput (2021). "Review on: Crimean Congo Hemorrhagic Fever." International Journal of Science and Innovative Research, IJESIR-NOVUS **02**(01).
Public Institute of Sweden, F., Professor Ali Mirazimi. (2017). "CCHFVaccine Project." from https://www.cchfvaccine.eu/projects.

Raabe, V. (2020). "Diagnostic Testing for CCHF.pdf." Journal of Clinical Microbiology **58**(4).

Rangunwala, A., R. R. Samudzi and F. J. Burt (2014). "Detection of IgG antibody against Crimean-Congo haemorrhagic fever virus using ELISA with recombinant nucleoprotein antigens from genetically diverse strains." Epidemiol Infect **142**(10): 2147-2154.

Rodriguez, S. E., D. W. Hawman, T. E. Sorvillo, T. J. O'Neal, B. H. Bird, L. L. Rodriguez, E. Bergeron, S. T. Nichol, J. M. Montgomery, C. F. Spiropoulou and J. R. Spengler (2022). "Immunobiology of Crimean-Congo hemorrhagic fever." Antiviral Res **199**: 105244.

Rusnak, J. M. (2011). "Experience with Ribavirin for Treatment and Postexposure Prophylaxis of Hemorrhagic Fever Viruses: Crimean Congo Hemorrhagic Fever, Lassa Fever, and Hantaviruses." Applied Biosafety **16**(2): 67-87.

Sahay, R. R., A. M. Shete, P. D. Yadav, S. Patil, T. Majumdar, R. Jain, D. A. Nyayanit, H. Kaushal, S. J. Panjwani, K. J. Upadhyay, C. L. Varevadiya, A. Vora, A. Kanani and R. R. Gangakhedkar (2021). "Sequential determination of viral load, humoral responses and phylogenetic analysis in fatal and non-fatal cases of Crimean-Congo hemorrhagic fever patients from Gujarat, India, 2019." PLOS Neglected Tropical Diseases **15**(8): e0009718.

Samudzi, R. R., P. A. Leman, J. T. Paweska, R. Swanepoel and F. J. Burt (2012). "Bacterial expression of Crimean-Congo hemorrhagic fever virus nucleoprotein and its evaluation as a diagnostic reagent in an indirect ELISA." J Virol Methods **179**(1): 70-76.

Sas, M. A., L. Comtet, F. Donnet, M. Mertens, Z. Vatansever, N. Tordo, P. Pourquier and M. H. Groschup (2018). "A novel double-antigen sandwich ELISA for the species-independent detection of Crimean-Congo hemorrhagic fever virus-specific antibodies." Antiviral Res **151**: 24-26.

Schulz, A., Y. Barry, F. Stoek, A. Ba, J. Schulz, M. L. Haki, M. A. Sas, B. A. Doumbia, P. Kirkland, M. Y. Bah, M. Eiden and M. H. Groschup (2021). "Crimean-Congo hemorrhagic fever virus antibody prevalence in Mauritanian livestock (cattle, goats, sheep and camels) is stratified by the animal's age." PLoS Negl Trop Dis **15**(4): e0009228.

Sisman, A. (2013). "Epidemiologic features and risk factors of Crimean-Congo hemorrhagic fever in Samsun province, Turkey." J Epidemiol **23**(2): 95-102.

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." Trop Med Infect Dis **5**(3).

Spengler, J. R., D. A. Bente, M. Bray, F. Burt, R. Hewson, G. Korukluoglu, A. Mirazimi, F. Weber and A. Papa (2018). "Second International Conference on Crimean-Congo Hemorrhagic Fever." Antiviral Res **150**: 137-147.

Spengler, J. R., Bente, Dennis A. (2017). "Crimean-Congo Hemorrhagic Fever in Spain - New Arrival or Silent Resident?" New England Journal of Medicine **377**(2): 106-108.

Spengler, J. R., E. Bergeron and P. E. Rollin (2016). "Seroepidemiological Studies of Crimean-Congo Hemorrhagic Fever Virus in Domestic and Wild Animals." PLoS Negl Trop Dis **10**(1): e0004210.

Spengler, J. R., A. Estrada-Pena, A. R. Garrison, C. Schmaljohn, C. F. Spiropoulou, E. Bergeron and D. A. Bente (2016). "A chronological review of experimental infection studies of the role of wild animals and livestock in the maintenance and transmission of Crimean-Congo hemorrhagic fever virus." Antiviral Res **135**: 31-47.

Temur, A. I., J. H. Kuhn, D. B. Pecor, D. A. Apanaskevich and M. Keshtkar-Jahromi (2021). "Epidemiology of Crimean-Congo Hemorrhagic Fever (CCHF) in Africa-Underestimated for Decades." Am J Trop Med Hyg **104**(6): 1978-1990.

Vawda, S., D. Goedhals, P. A. Bester and F. Burt (2018). "Seroepidemiologic Survey of Crimean-Congo Hemorrhagic Fever Virus in Selected Risk Groups, South Africa." Emerging infectious diseases **24**(7): 1360-1363.

Wampande, E. M., P. Waiswa, D. J. Allen, R. Hewson, S. D. W. Frost and S. C. B. Stubbs (2021). "Phylogenetic Characterization of Crimean-Congo Hemorrhagic Fever Virus Detected in African Blue Ticks Feeding on Cattle in a Ugandan Abattoir." Microorganisms **9**(2).

Whitehouse, C. A. (2004). "Crimean-Congo hemorrhagic fever." Antiviral Res **64**(3): 145-160.

Wilson, M. L., B. LeGuenno, M. Guillaud, D. Desoutter, J. P. Gonzalez and J. L. Camicas (1990). "Distribution of Crimean-Congo hemorrhagic fever viral antibody in Senegal: environmental and vectorial correlates." Am J Trop Med Hyg **43**(5): 557-566.

Zivalioglu, M. (2008). "The determinations of epidemiologic features of Crimean-Congo haemorrhagic fever." National Thesis, 19 Mayis University Faculty of Medicine, Samsun.

Zivcec, M., F. E. Scholte, C. F. Spiropoulou, J. R. Spengler and E. Bergeron (2016). "Molecular Insights into Crimean-Congo Hemorrhagic Fever Virus." Viruses **8**(4): 106.

Zouaghi, K., A. Bouattour, H. Aounallah, R. Surtees, E. Krause, J. Michel, A. Mamlouk, A. Nitsche and Y. M'ghirbi (2021). "First Serological Evidence of Crimean-Congo Hemorrhagic Fever Virus and Rift Valley Fever Virus in Ruminants in Tunisia." Pathogens **10**(6): 769.