Understanding Human Exposure to Viral Haemorrhagic Fevers in Uganda: Occupational, Behavioural, and Ecological Factors

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Abstract

Introduction

Uganda experiences frequent outbreaks of viral haemorrhagic fever viruses (VHFVs), placing healthcare workers (HCWs) and local communities at risk of exposure. Among these, Crimean Congo haemorrhagic fever virus (CCHFV) is a tick-borne zoonotic pathogen that can cause severe haemorrhagic disease with high fatality rates among hospitalised cases. Despite a rise in reported infections over the past decade, the true burden of CCHFV remains underestimated due to mild or misdiagnosed presentations. Understanding the complex interplay of occupational, behavioural, and ecological risk factors is essential for identifying high-risk populations and guiding effective interventions. Previous research has highlighted the importance of geographic variability in exposure risk, yet socioecological determinants remain poorly understood. This thesis aims to address these gaps and increase the knowledge around VHFVs with a focus on CCHFV.

Methods

The body of work is based on three cohort studies designed to investigate exposure to CCHFV and other VHFVs in Uganda. Firstly, a case-control study was conducted among 639 HCWs and 714 age- and sex-matched community members, to understand occupational risk for VHFV exposure. The study sites

comprised hospitals in Gulu, Arua and Kasese districts of Uganda. Serum was tested for Ebola virus (EBOV) and CCHFV seropositivity by ELISA and for Rift Valley fever virus (RVFV) by indirect immunofluorescence. Exposure risk factors were evaluated with a structured survey and analysed by multivariable logistic regression.

A qualitative investigation was next carried out to study human-animal-tick interactions through 24 focus group discussions (FGDs) and 31 key informant interviews (KIIs), in six environmentally and socioecologically diverse districts of Uganda. FGDs were conducted in groups of community leaders, men, women and teenagers. Medical doctors, veterinarians, traditional healers, district surveillance officers, and herdsmen were also interviewed as key informants. Data were translated into English, transcribed, and analysed using iterative categorisation.

The final quantitative cohort study used an analytical framework to estimate seroprevalence in the first four of six selected districts of Uganda as part of an interim analysis of the wider AVI study. 1,059 participants were recruited through multi-level randomisation and stratified by age. Serum samples were collected from each participant, and a structured survey was performed, which was informed by the preceding qualitative research. CCHFV antibody testing was carried out to estimate CCHFV exposure and force of infection (FOI) . Multivariable logistic regression was used to evaluate underlying risk factors.

Results

Overall, seropositivity in the HCWs study was 16% for EBOV, 19% for CCHFV, and 2% for RVFV seropositivity. The highest odds of exposure were noted in Arua district for both EBOV (AOR = 9.01 [95% CI = 5.48-15.4]) and CCHFV (AOR = 4.67 [95% CI = 3.11-7.13]), around hospitals that had no previously documented cases of VHFVs. Overall, HCWs had a lower odds of EBOV ex-

posure than community members (AOR = 0.37 [95% CI0.26-0.51]), as well as of CCHFV exposure (AOR = 0.42 [95% CI 0.31-0.57]). Homemakers and cleaners had the highest seropositivity for EBOV and CCHFV in the respective study groups.

Thirteen district clusters showed notable differences in climate, land use, proximity to wildlife, and subregional locations within Uganda. Six of these were selected for subsequent qualitative and quantitative cohort studies. Participants from both FGDs and KIIs described distinct living conditions and practices, highlighting regional variation.

The majority of the people that we interviewed as part of our qualitative study experienced tick bites, some as frequently as every day. Close contact with animals was common, including cohabitation, largely due to concerns about animal theft. Less frequent but notable practices included slaughtering animals for consumption or sacrifice, drinking blood, and interactions with wild animals during hunting. Slaughtering and butchering were reported if an animal was unwell or had died. Plucking and roasting engorged ticks for consumption was a practice described in the Kaabong and Arua districts of Northern Uganda.

The quantitative study highlighted varying estimated seroprevalence to CCHFV, ranging from 2.2% in Kaabong district to 18.2% in Kasese district. A multivariable analysis, including known risk factors for CCHFV transmission, revealed significant differences in CCHFV seropositivity between study locations ($p = 0.002$) and age groups ($p < 0.001$). The FOI showed an accumulation of seropositivity with age, suggesting constant exposure rather than isolated outbreaks.

Discussion

This PhD demonstrates that exposure to VHFVs in Uganda is extremely high, and is shaped by a complex interplay of ecological, occupational, and behavioural factors. In the HCWs study, seropositivity was highest for CCHFV (19%), followed by EBOV (16%) and RVFV (2%). The unexpectedly high odds of exposure in Arua district, where CCHFV has only very rarely been reported, strongly suggests the presence of mild and/or misdiagnosed cases. Elevated risk among homemakers and cleaners, within community members and HCWs respectively, points to occupational exposures that have been largely overlooked. Qualitative findings, including daily tick bites, animal cohabitation, and practices such as tick collection for consumption, underscore the need for context-specific evaluation of risk behaviours in Uganda's diverse settings. These behaviours represent possible transmission routes for CCHFV and highlight the importance of future studies to quantify their contribution to infection risk, and to identify targeted and culturally appropriate interventions. The serosurvey revealed significant variation in estimated seroprevalence across surveyed districts (ranging between 2.2% and 18.2%), reinforcing previous findings that study location and, therefore, environmental and geographic factors are key drivers of exposure to CCHFV. These insights can support the identification of high-risk regions and guide targeted control strategies for CCHFV transmission, including the implementation of tick control measures and the prioritisation of future vaccine trials.

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Acknowledgements

I never imagined myself ever reaching this point when I first started to learn about science in school. I am so grateful for all the direct and indirect support and encouragement from colleagues, mentors, supervisors, friends and family. I want to take the following pages to thank everyone individually. However, there were countless people who might have had only a small impact on me and my work, but without them, I might not be here. This section is only a recollection of my memories, and it is by no means complete.

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and occasional hard time as I expected it. I had incredible help, not only by my official supervisory team, but also from others who stepped in when I needed guidance on a new topic or a whole new field. This includes Prof. Lucy Pickering, Lazaaro Mujumbusi and Janet Seeley, who introduced me to social sciences, and taught me how to plan, conduct and analyse FGDs and KIIs. Prof. Paul Johnson, who had an open ear for any statistical problem I encountered. Last but not least, Prof. Kimberly Fornace and Emilia Johnson, who introduced and continued to support me with spatial datasets, environmental variables, and clustered study designs.

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Author's Declaration

I, Marina Kugler, declare that, except where explicit reference is made to the contribution of others, this thesis is the result of my work and has not been submitted for any other degree at the University of Glasgow or any other institution.

The use of AI

I acknowledge the use of Artificial Intelligence (AI) assistance during the process of my PhD. Grammarly Premium (Grammarly Inc.; https://www.grammarly.com) was used as a tool to help me identify spelling mistakes and simple grammatical errors. As a non-mother-tongue English speaker, this was a great help and enabled me to focus on the science without too much distraction about the correct wording.

I also used Copilot (Microsoft Corporation; https://copilot.microsoft.com) in certain aspects of my work. As it was only available from around 2024, all work on Chapter 2 and 3 was completed without the use of any AI. In the later Chapters 4 and 5, it was used like a search machine asking for specific questions, and answers were always checked for correctness by visiting the sources. Especially for the analysis in Chapter 5, it was often used to facilitate and speed up improving and identifying errors in R code. Copilot helped me write my thesis like a mentor who can listen day and night. It supported me

in refining sentence structure and grammar. All content, interpretations, and final proofreading are entirely my own work.

Abbreviations

adjusted gVIF	adjusted generalised variance inflation factor
AI	Artificial Intelligence
AIC	Akaike Information Criterion
AVI study	ArboViral Infection study
BDBV	Bundibugyo virus
CAO	Chief Administrative Officer
CCHF	Crimean-Congo haemorrhagic fever
CCHFV	Crimean-Congo haemorrhagic fever virus
CVR	MRC-University of Glasgow Centre for Virus Research
DENV	dengue virus
DHO	District Health Officer
DRC	Democratic Republic of the Congo
DVO	District Veterinary Officer
EBOV	Ebola virus
ELISA	enzyme-linked immunosorbent assays
ER	endoplasmic reticulum
FCDO	Foreign, Commonwealth and Development Office
FGD	focus group discussion

FGDs	focus group discussions
FOI	force of infection
GDP	gross domestic production
glm	generalised linear model
glmm	generalised linear mixed model
GP	glycoprotein
$GP_{1,2}$	$glycoprotein_{1,2}$
GPC	glycoprotein precursor
HCWs	healthcare workers
IC	Iterative categorisation
KAP	knowledge, attitude and practices
KIIs	key informant interviews
LEK	local ecological knowledge
MARV	Marburg virus
NP	nucleoprotein
ODs	Optical Density values
ORs	odds ratios
PPE	personal protective equipment
RVFV	Rift Valley fever virus
sq km	square kilometres
sq mi	square miles
SUDV	Sudan virus
UNCST	Uganda National Council for Science and Technology

UVRI	Uganda Virus Research Institute
UVRI REC	Uganda Virus Research Institute Research Ethics Committee
VHF	viral haemorrhagic fever
VHFV	viral haemorrhagic fever virus
VHFVs	viral haemorrhagic fever viruses
VHTs	village health teams
VIFs	variance inflation factors
WSS	total within-cluster sum of squares
YFV	yellow fever virus

Chapter 1

Introduction

1.1 Background

Viral haemorrhagic fever viruses (VHFVs) are viruses of high concern due to their high transmissibility, morbidity and mortality. They are a group of diverse zoonotic viruses, all of which have the propensity to cause fever and haemorrhagic illness. Crimean-Congo haemorrhagic fever virus (CCHFV) was the main focus of this PhD, transmitted to humans by ticks or from contact with infected animal blood or tissue. Human-to-human transmission may also occur as a result of direct contact with bodily fluids (Tsergouli *et al.* 2020).

To gain a deeper insight into exposure to VHFVs in general and specifically CCHFV in more detail, three studies were employed to investigate how occupational roles, personal behaviours, and the socioecological context influence the risk of human exposure to VHFVs in Uganda. This PhD includes Uganda's first cross-sectional, household-based, randomised, seroepidemiological survey of CCHFV exposure. It provided a unique opportunity to analyse risk behaviours and to better understand the current burden of CCHFV in Uganda.

Uganda is a landlocked country in East Africa and is one of the most biodiverse

locations on the planet. Its cultural diversity includes several distinct tribes and languages. Uganda has benefited from relative political stability and has invested significantly in medical research. It was the first African country with publications on CCHFV research, and the first to register CCHFV in the Catalogue of Arthropod-borne Viruses of the World (Hoogstraal, 1979). The presence of sporadic reported cases and the unknown overall infection rate in the country formed the motivation to carry out this research. I aimed to characterise the seroprevalence in the country and to investigate underlying risk factors. Within this introductory chapter, I introduce Uganda as the setting for the studies within this PhD, and I introduce VHFVs within the Ugandan context. I end by highlighting the research gaps identified, my aims and hypotheses, and describing the thesis outline.

1.2 Uganda

The Republic of Uganda is a country in East Africa, bordering Kenya to the east, South Sudan to the north, the Democratic Republic of the Congo (DRC) to the west, Rwanda to the southwest and Tanzania to the south (Figure 1.1). A substantial part of the border with Tanzania and Kenya lies on Lake Victoria.

The country comprises around 200,000 square kilometres (sq km) (77,220 square miles (sq mi)) of land, which is on average 900 meters above sea level. It is located between 1° S and 4° N latitude, and between 30° E and 35° E longitude.

Lake Victoria is the largest of the five lakes within Uganda (Figure 1.2), which form part of the East African Rift System. The other four are Lake Kyoga, Lake Albert, Lake Edward and Lake George, of which Lake Albert and Lake Edward are shared with the DRC. The smaller arm of the River Nile, the White Nile, originates in Uganda as the Victoria Nile and is fed by all the large Lakes.

The highest mountains in Uganda lie within the Rwenzori Mountain range on the western border, and Mount Elgon on the eastern border.

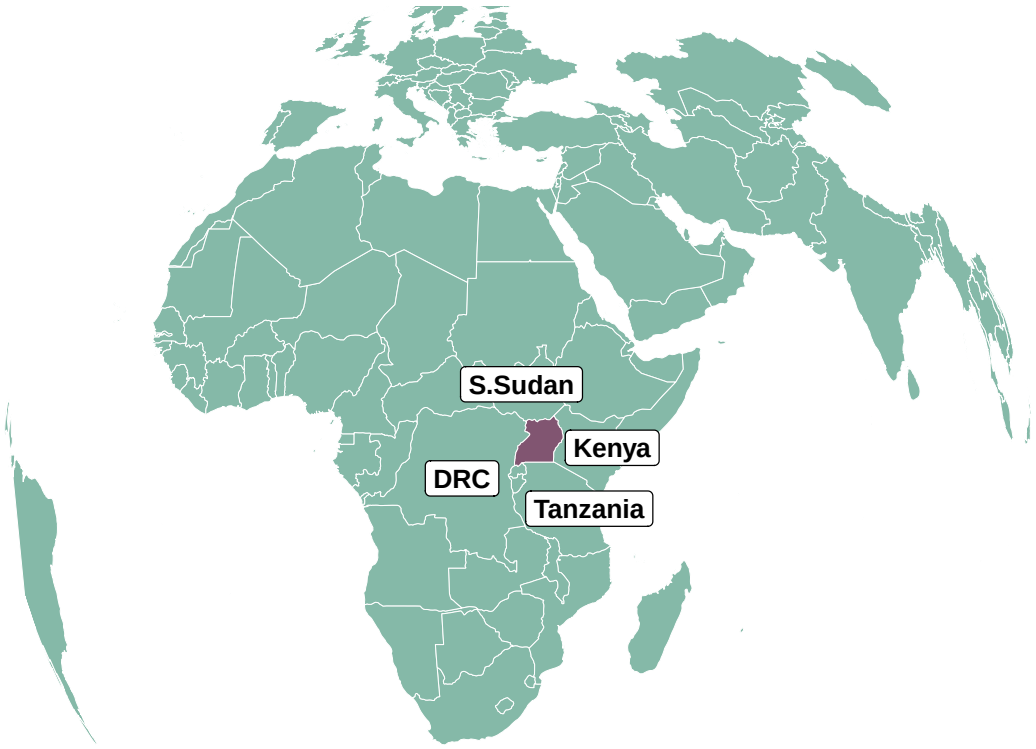


Figure 1.1: **World map** highlighting Uganda and neighbouring countries (S.Sudan, Kenya, Tanzania, Rwanda, Democratic Republic of the Congo DRC).

Most of Uganda has a tropical savannah climate, with areas of tropical rainforest and tropical monsoon climate, as classified using the Koeppen-Geiger system (Beck *et al.* 2018; Koeppen, 1884), which is based on monthly air temperature and precipitation recordings and seasonality.

Large areas of the country are protected from human settlement, including ten national parks. The largest national parks are Murchison Falls and Queen Elizabeth National Park in the western and southwestern parts of the country.

Uganda consists of four Regions (Central, Western, Eastern and Northern), which are divided into districts. In July 2020, there were 135 districts plus Kampala, the capital city. Between 2020 and 2025, more districts were created, and some towns were elevated to city status (Kanyere, 2025). In this thesis,

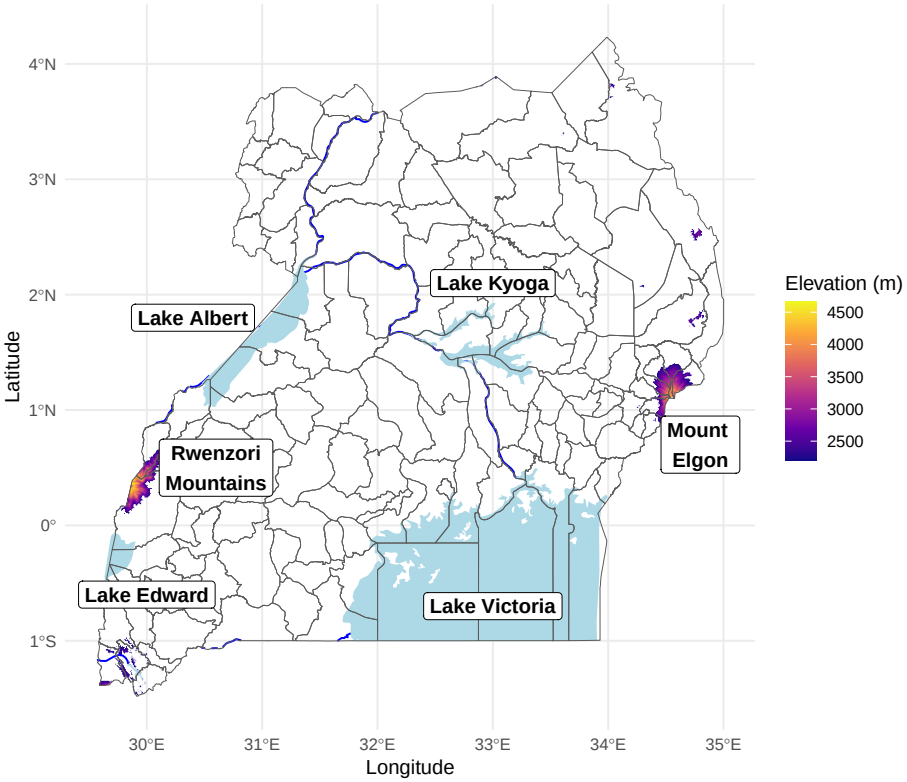


Figure 1.2: **Uganda map** showing the four largest lakes, the Rwenzori mountains and Mount Elgon, and the largest rivers. Elevation above 2200 m is highlighted on the map.

I have used the 136 districts (including the city of Kampala) as of 2020 to maintain consistency throughout the work.

45.9 million people lived in Uganda as counted in the National Census of 2024 (Uganda Bureau of Statistics, 2024). The country comprises more than 60 ethnic groups, the largest being Baganda (located around the Central region), Banyankole (in the southwestern area), and Basoga and Iteso (within the Eastern Region). The country is very young; around 50% of the population is aged 18 years or younger. Poverty is a significant problem, with 30.7% of people in 2024 experiencing severe food insecurity (Uganda Bureau of Statistics, 2024).

The health system in Uganda is decentralised, meaning that the first point of care is usually a level I health centre, rising to health centre IV, and lastly the national referral hospital in Kampala (Mulago National Referral Hospital). State health facilities are free of charge. However, it is common for patients and their families to pay for medical equipment, medicines, food, and linen. Uganda has no national health insurance system. Only 1.1% of the population has any type of health insurance policy, and this is more common in urban than rural areas (Uganda Bureau of Statistics, 2024). Uganda faces high prevalences of serious infectious diseases. Common viral infections include HIV/AIDS, Hepatitis A and E, rotavirus and other gastrointestinal pathogens. Uganda has several viral haemorrhagic fever virus (VHFV) pathogens, including caused by Ebola virus (EBOV), Sudan virus (SUDV), Marburg virus (MARV), Bundibugyo virus (BDBV), yellow fever virus (YFV), dengue virus (DENV), Rift Valley fever virus (RVFV) and CCHFV (WHO, 2023).

1.3 Viral haemorrhagic fever viruses

VHFVs are a diverse group of viruses from many viral families, including the *Arenaviridae*, *Filoviridae*, *Flaviviridae*, *Hantaviridae*, *Nairoviridae*, *Peribun-*

yaviridae, and *Phenuiviridae* (Hewson, 2024). All VHFVs are enveloped RNA viruses, and all VHFVs are zoonotic viruses. Transmission to humans occurs either through direct contact with infected animals or via haematophagous arthropods, including mosquitoes (eg RVFV) and ticks (eg CCHFV) (Hewson, 2024).

Viral haemorrhagic fever (VHF) is defined by the characteristic symptoms of fever and bleeding, although it is increasingly clear that bleeding is not always a feature of infection with VHFVs (Mcelroy, 2015). VHF illnesses can, in fact, vary in clinical presentation and may present with a spectrum from relatively mild to severe and life-threatening disease. They may cause multiple organ failure, and abnormal vascular regulation and vascular damage may manifest with hypotension, flushing of the skin, and vasodilation of the conjunctivae as well as overt bleeding (Paessler & Walker, 2013). Severe disease is less common with some viruses, for example RVFV and possibly CCHFV, and more common in others, including Marburg virus disease or Ebola virus disease, where case fatality rates can reach up to 80% (Belhadi *et al.* 2022). Management of VHFV outbreaks is mostly limited to isolation and supportive care, combined with contact tracing and ring vaccination for Ebola virus disease (Belhadi *et al.* 2022; Henao-Restrepo *et al.* 2017). Ebola virus disease can also now be treated with antivirals, including remdesivir and monoclonal antibody therapy (Mulangu *et al.* 2019; WHO, 2022).

VHFVs are found on every continent except Antarctica and are widespread in Africa, Asia and South America. Some viruses are confined to smaller areas, such as BDBV (only ever reported in Uganda and DRC) and others, such as CCHFV, have been reported on three continents, namely Asia, Africa and Europe.

VHFVs are a global health concern for multiple reasons. Firstly, they have the potential for rapid outbreaks, as seen in the 2013-2016 West African Ebola virus outbreak, which included case importations to countries initially unaf-

fected (WHO Ebola Response Team, 2016). Secondly, there are limited treatment options and high associated mortality rates.

In Uganda, the VHFVs of highest concern are the viruses in the *Filoviridae* family, including EBOV, SUDV, BDBV and MARV; CCHFV in the family of *Nairoviridae*; YFV and DENV within the *Flaviviridae*; and RVFV in the *Phenuiviridae*. Since 2010, there has been a VHFV surveillance and laboratory programme at the Uganda Virus Research Institute (UVRI) in Entebbe, reporting to the Uganda Ministry of Health (Shoemaker *et al.* 2018). The focus in this thesis is on CCHFV, EBOV, and RVFV, which are introduced separately in the following sections.

1.3.1 Crimean-Congo haemorrhagic fever virus (CCHFV)

CCHFV is a member of the *Nairoviridae* family and in the genus *Orthonairovirus* with the species name *Orthonairovirus haemorrhagiae*. It falls in the order *Hareavirales* and the class *Bunyaviricetes* (Kuhn *et al.* 2024). It has a negative-sensed, single-stranded RNA genome, which is segmented and consists of L, M and S segments (see Figure 1.3). Genomic RNA is bound to the viral nucleoprotein and to the viral RNA-dependent RNA-polymerase L, forming the ribonucleoprotein complex. The virus particle is enveloped with a round shape and contains the Gn and Gc surface glycoproteins embedded within the membrane, which are responsible for receptor binding and viral entry (Bente *et al.* 2013; Hawman & Feldmann, 2023). Further viral proteins of CCHFV are the small non-structural protein (NSs; triggers cell apoptosis), and multiple further proteins all derived from the precursor (glycoprotein precursor (GPC)) of Gn and Gc protein (GP160/85 (excreted), GP38 and the medium non-structural protein NSm).

Virus entry occurs through Gn and Gc, after clathrin-dependent and pH-dependent fusion of the virus membrane with the cell membrane. Virus recep-

tor candidates/essential entry factors include nucleolin, DC-SIGN, low-density lipoprotein receptor (LDLR), apolipoprotein E (ApoE) and the soluble milk fat globule-EGF factor 8 protein (MFGE8) (Ma *et al.* 2025). Replication and translation occur within the cytoplasm. The GPC is translated into endoplasmic reticulum (ER) and processed within the ER and Golgi apparatus into the individual proteins. New genomes are packed into the Golgi apparatus, and new virus particles are released via the secretory pathway (Hawman & Feldmann, 2023). In mouse models, the primary targets of CCHFV infection were found to be hepatocytes and endothelial cells (Hawman & Feldmann, 2023).

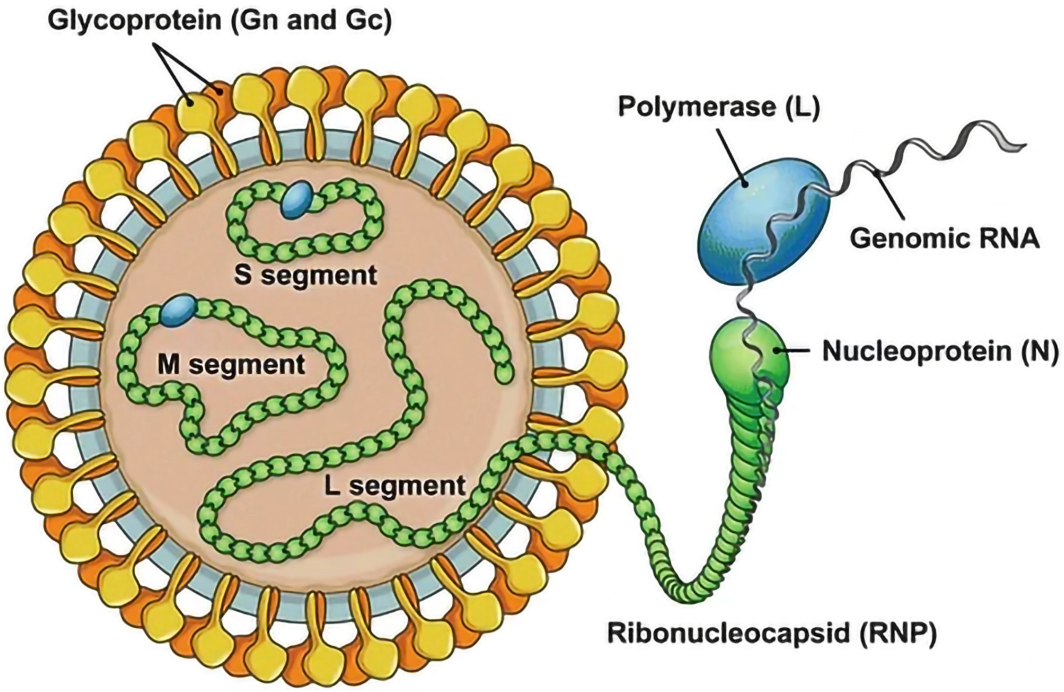


Figure 1.3: ***Hareavirales* order structure.** The order *Hareavirales* includes the family *Phenuviridae* (including RVFV) and the family *Nairoviridae* (including CCHFV). Image adapted from (Whitehouse *et al.* 2015) using Adobe Photoshop (Adobe Inc., 2025). The membrane-bound glycosylated proteins Gn and Gc, and the three RNA viral genome segments bound with nucleoprotein and L protein are shown.

Most human infections with CCHFV result in a mild, non-specific febrile illness (Bente *et al.* 2013). Bodur *et al.* 2012 estimated in their study in Turkey that 88% of cases are subclinical, and modelling efforts by Vesga, Métras, *et*

al. 2022 in Afghanistan suggested that 69% of cases are subclinical. However, some patients develop a severe haemorrhagic disease, characterised by bleeding into the skin, bleeding from gastrointestinal and urinary tracts, hepatomegaly and splenomegaly, which can lead to death (Bente *et al.* 2013). Case fatality rates vary (Belobo *et al.* 2021), with a study by Balinandi *et al.* 2022 reporting 31.2% mortality in patients hospitalised with CCHFV in Uganda between 2013 and 2019.

Treatment is currently focused on supportive measures (Gholizadeh *et al.* 2022). Multiple antivirals are in development and pre-clinical stages (including nucleoside analogues, polymerase inhibitors and monoclonal antibodies), but with minimal data on clinical efficacy to date (Hawman & Feldmann, 2023). CCHFV vaccine candidates are also in development, including mRNA and adenovirus vectored vaccines, but none have yet been approved for use (Ahata & Akçapınar, 2023; Hawman & Feldmann, 2023).

The main transmission route of CCHFV to humans occurs through bites of infected ticks (Hawman & Feldmann, 2023) (Figure 1.4), described in more detail below in the subsection: "Tick vectors of CCHFV". Ticks also regularly bite domestic and wild mammals for their bloodmeal, and can transmit the virus in the process.

Mammals (other than humans) develop a short-lasting viremia but experience no symptoms or only mild illnesses (Spengler, Estrada-Peña, *et al.* 2016). Exposure to CCHFV has been detected in various domestic (cattle, goats, sheep, and dogs) and wild mammals (Celina *et al.* 2024). Humans can also get infected when contacting contaminated blood or animal products (Hawman & Feldmann, 2023). A case study of an infection cluster triggered by the consumption of an uncooked sheep liver indicated that exposure to raw or undercooked animal meat is also a risk factor (Sharifi Mood *et al.* 2011).

Human-to-human transmission has been well reported in the context of nosoco-

mial infections (Leblebicioglu *et al.* 2016; Pshenichnaya & Nenadskaya, 2015; Tsergouli *et al.* 2020), possible horizontal transmission from mother to child (Saijo *et al.* 2004), and sexual transmission (Ergonul & Battal, 2014; Karbalalaei & Keikha, 2022; Pshenichnaya *et al.* 2016). However, large outbreaks, with extensive human-to-human transmission chains, as seen commonly in Ebola disease, have not been reported (Hawman & Feldmann, 2023).

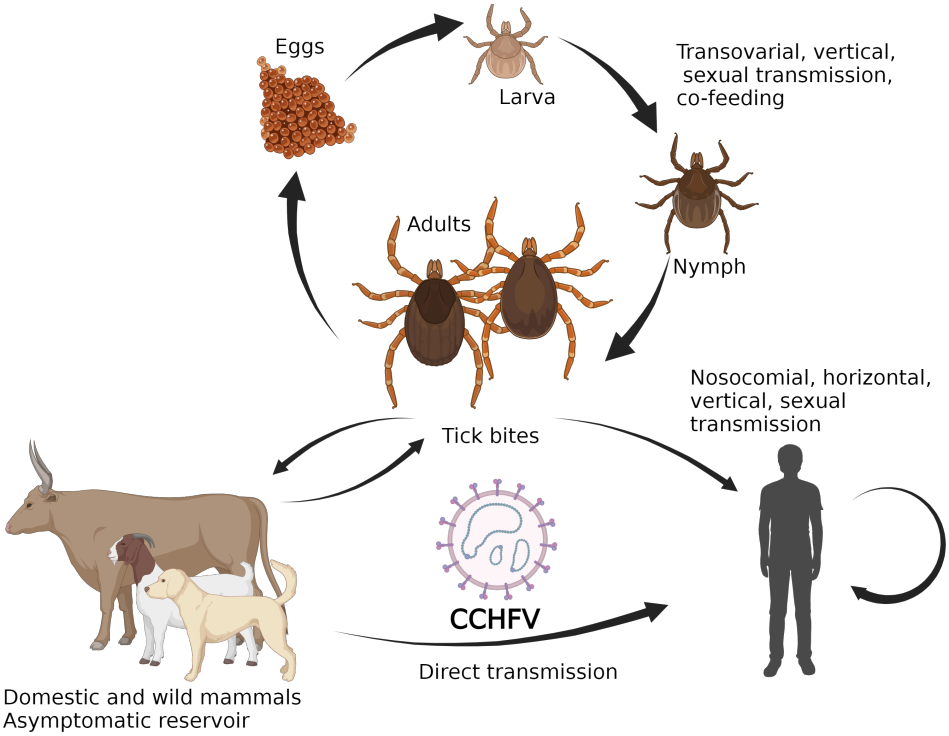


Figure 1.4: **Simplified CCHFV transmission.** Created in https://BioRender.com. Humans and domestic and wild mammals may be infected with CCHFV through the bite of an infected tick. Ticks of the species *Hyalomma*, *Amblyomma*, and *Rhiphacephalus* have been found to have detectable CCHFV RNA in Uganda. For *Hyalomma* ticks, it has been shown that they are competent to transmit the virus through transovarial, vertical, and sexual routes, and through co-feeding. Humans may also be infected by direct contact with infected animal blood or tissues, or following contact with a sick human or their body fluids.

CCHFV has existed for millennia, with case reports from multiple historical sources (the first from Tadjikistan in the 12th century) and a geographically

wide distribution of both the virus and vector species (Bente *et al.* 2013; Hoogstraal, 1979). During a large epidemic in 1944-1945 in Crimea, Chumakov *et al.* suggested the viral nature of "Crimean haemorrhagic fever" and identified ticks as possible vectors (Hoogstraal, 1979). In 1967, the virus was isolated by inoculating infected blood into newborn white mice by Chumakov and Woodall *et al.* 1967. Woodall *et al.* 1967 worked with isolates described in Simpson *et al.* 1967, with the earliest from 1956 (a boy from Stanleyville in the former Belgian Congo), multiple sporadic cases from Uganda (Wakiso, Arua, Kampala and Kigezi district), and multiple laboratory-inquired infections at the East African Virus Research Institute in Entebbe (now UVRI). Casals, 1969 received the isolated virus from Chumakov and Woodall, and conducted agar gel precipitation and neutralisation tests. They stated in 1969 that the viruses investigated were "antigenically indistinguishable" (Casals, 1969), and suggested the new name "Crimean haemorrhagic fever - Congo (CHF-Congo)". Hoogstraal, 1979 employed the new name "Crimean-Congo haemorrhagic fever", as used now.

CCHFV is widespread on the Asian and African continents, with multiple countries reporting cases since the 1970s (Bente *et al.* 2013; Ergonul, 2006). Further studies have identified a wider distribution based on serological studies in domestic and wild animals (Celina *et al.* 2024).

Uganda experiences sporadic outbreaks of CCHFV (Balinandi *et al.* 2022; Mirembe *et al.* 2021) (presented in Figure 1.5). These have been systematically recorded under the VHF surveillance system since 2010, which comprises 20 sentinel surveillance sites across Uganda (Shoemaker *et al.* 2018). However, cases are still frequently misdiagnosed as malaria or bacterial infection unless investigated during an outbreak of another VHFV. For example, during a five months SUDV outbreak in 2022, 13 CCHFV cases from 10 different districts were detected (Balinandi *et al.* 2024). Suspect cases for SUDV were not only tested for SUDV but also other haemorrhagic fever viruses, including CCHFV,

leading to an unusually high number of detected cases within a short period of time (Balinandi *et al.* 2024; Kabami *et al.* 2024; Muleme *et al.* 2017). Between 2013 and 2019 the average number of cases for CCHFV per year was 4.7 (Balinandi *et al.* 2022). We have also detected undiagnosed CCHFV cases in the endemic setting in two separate studies (Ashraf *et al.* 2025). These observations highlight the need to screen for VHFVs outside formal outbreaks. A single case of CCHFV in Uganda is considered an outbreak, and is treated by the Ministry of Health with urgency (Formenty *et al.* 2007). However, not every district has adequate preparations in place (Zalwango *et al.* 2024). The cattle corridor, stretching from southwestern to northeastern Uganda, is dominated by pastoral rangelands and is therefore considered a high-risk area of CCHFV transmission. Most recognised cases have been reported from within this area (Mirembe *et al.* 2021), however, more recent cases have been detected outside, likely as a result of increased awareness of disease risk, including as a result of this work (Balinandi *et al.* 2022).

Tick vectors of CCHFV

As mentioned above, ticks are thought to be the predominant route of CCHFV transmission to humans (Hawman & Feldmann, 2023). Chumakov *et al.* suggested in the large outbreak of 1944 - 1945, that ticks of the species *Hyalomma marginatum* were the primary vector of the virus (Hoogstraal, 1979; Linthicum & Bailey, 2023). *Hyalomma* ticks have since then been commonly found to be infected with CCHFV and competent vectors for CCHFV. Further genus and species of ticks have since then been found to carry CCHFV, specifically Amblyomma and Rhipicephalus in Uganda (Atim, Ashraf, *et al.* 2023; Celina *et al.* 2024; Gargili *et al.* 2017).

Once ticks are infected, they remain infected for life, and they transmit the virus transovarially, sexually, and through co-feeding to further generations and other ticks (Gargili *et al.* 2017; Shepherd *et al.* 1991).

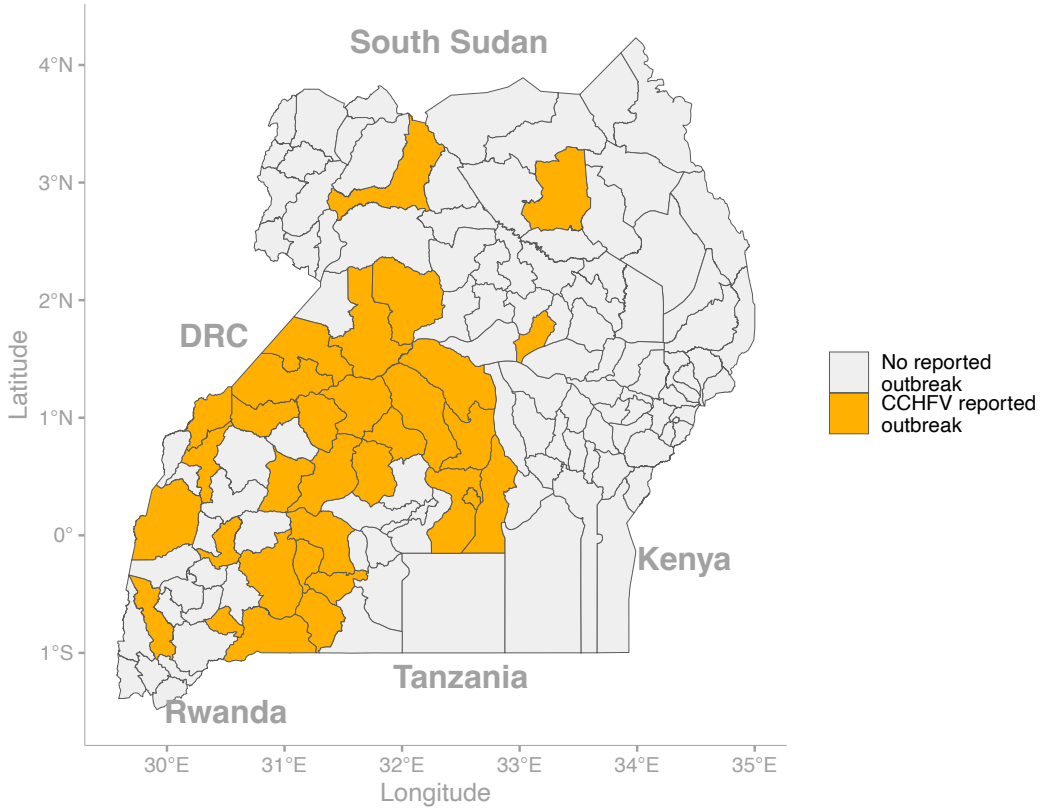


Figure 1.5: **Map of Uganda presenting previous CCHFV outbreaks.** Data on outbreaks available between 2000 and 2024 from (Balinandi *et al.* 2022) and personal communication (Balinandi S).

Multiple research groups have modelled environmental drivers of tick presence and activity, to predict CCHFV cases (Celina *et al.* 2023; Ilboudo, Oloo, *et al.* 2025; Lule *et al.* 2022; Messina *et al.* 2015; Okely *et al.* 2020; Vesga, Clark, *et al.* 2022). Variables like soil temperature, isothermality, precipitation, relative humidity, saturation deficit, normalised difference vegetation index, bare soil cover, slope, and many more were used in these models. However, spatial resolution for Uganda at a country level shows no clear area of high risk for CCHFV transmitting ticks or infection risk for CCHFV, as cases have occurred widely and multiple ticks are present in different parts of the country. A more detailed within-country analysis was identified as a research gap in this work.

1.3.2 Ebola virus (EBOV)

EBOV is a member of the *Filoviridae* family within the genus *Orthoebolavirus*, together with SUDV, BDBV and MARV. It has a negative-sense, single-stranded, non-segmented RNA genome, and an enveloped and filamentous shape. It encodes seven structural and two soluble proteins. The nucleoprotein (NP), VP35, VP24, VP30 and the viral polymerase L, together with the viral RNA, make up the nucleocapsid. A matrix layer of VP40 surrounds the nucleocapsid, and within the viral membrane lies a trimeric glycoprotein$_{1,2}$ (GP$_{1,2}$) (Bodmer *et al.* 2024). A visual representation is shown in Figure 1.6.

The lifecycle of orthoebolaviruses takes place entirely in the cytoplasm of infected cells. Attachment depends mainly on the viral GP$_{1,2}$. The virus attaches to the Niemann-Pick CI receptor, C-type lectins, phosphatidylserine-binding receptors, and antibody-dependent enhancement may also play a role in attachment and entry to the cell (Bodmer *et al.* 2024). After attachment, the virus particle is taken up via macropinocytosis, and the host and virus membranes fuse together to release the nucleocapsid. Transcription and translation occur within the cytoplasm to produce viral proteins. The GP$_{1,2}$ is processed in the endoplasmic reticulum and Golgi network. The genome replicates within

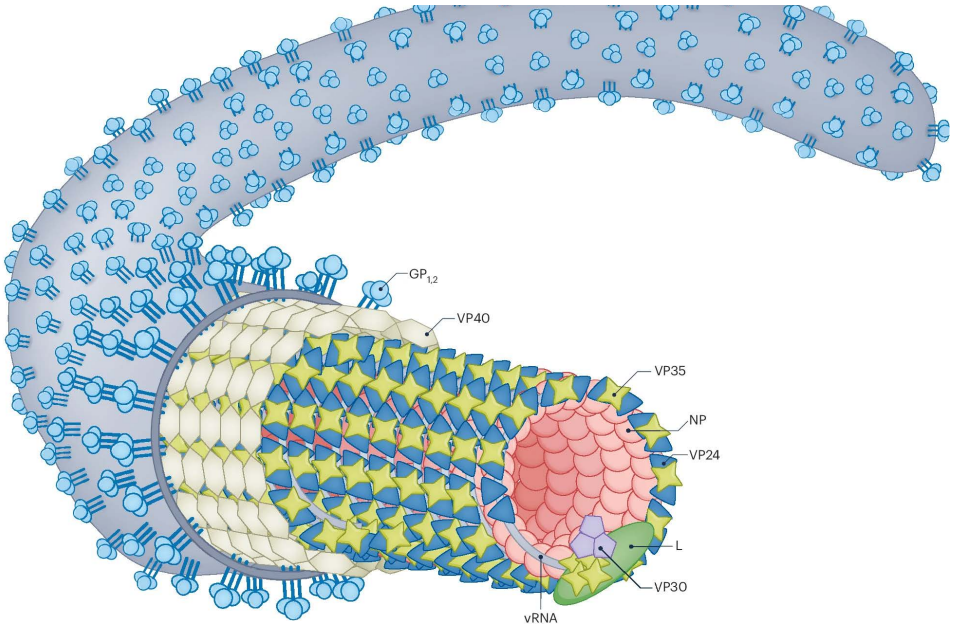


Figure 1.6: ***Orthoebolavirus* particle structure.** Adapted from Bodmer *et al.* 2024, presenting the viral genome (vRNA, grey), which is packed by the nucleoprotein (NP, red). Together with the VP30, VP35, VP24, and the viral polymerase L, they make up the nucleocapsid. VP40 surrounds the nucleocapsid, and the glycoprotein ($GP_{1,2}$, light blue) lies within the viral membrane. ELISAs targeting antibodies against NP and $GP_{1,2}$ were conducted in Chapter 2.

inclusion bodies and is then transported to the plasma membrane to be incorporated into newly formed virus particles by viral budding (Bodmer *et al.* 2024). The main initial target cells of *orthoebolaviruses* are antigen-presenting cells, including monocytes, macrophages, and dendritic cells. The virus is then transported through the lymphatic system to the liver and spleen and can replicate in a wide range of cell types (Feldmann & Geisbert, 2011).

The early symptoms of EBOV include fever, fatigue, loss of appetite, vomiting, diarrhoea, headache, and abdominal pain (WHO Ebola Response Team, 2014). These can develop into severe disease with haemorrhage, tachypnoea, anuria and shock, and ultimately death in a large percentage of patients (Paessler & Walker, 2013). The case-fatality rate varies by virus species, outbreak and healthcare management, from around 40% to 90% (Feldmann & Geisbert, 2011; WHO Ebola Response Team, 2016).

The WHO recommends two monoclonal antibodies targeting the viral GP (mAb114 and REGN-EB3) as treatment options for EBOV (Mulangu *et al.* 2019; WHO, 2022).

Two licensed vaccines are available for EBOV: rVSV-ZEBOV-GP (only EBOV) and Ad26.ZEBOV MVA-BN-Filo boost (multivalent) vaccine. The effectiveness of newer vaccines directed against MARV and SUDV is under current evaluation (Malik *et al.* 2023).

Most patients with EBOV are infected by direct contact with body fluids or direct contact with patients or cadavers (Feldmann & Geisbert, 2011). Infectious particles are present in sweat, blood, and semen. Outbreaks are thought to start with a zoonotic origin, for example, following the butchering of an infected chimpanzee, or handling and consumption of bats (Feldmann & Geisbert, 2011; Koch *et al.* 2020). A proposed transmission cycle is presented in Figure 1.7.

The first recorded human outbreaks with orthoebolaviruses occurred in 1976

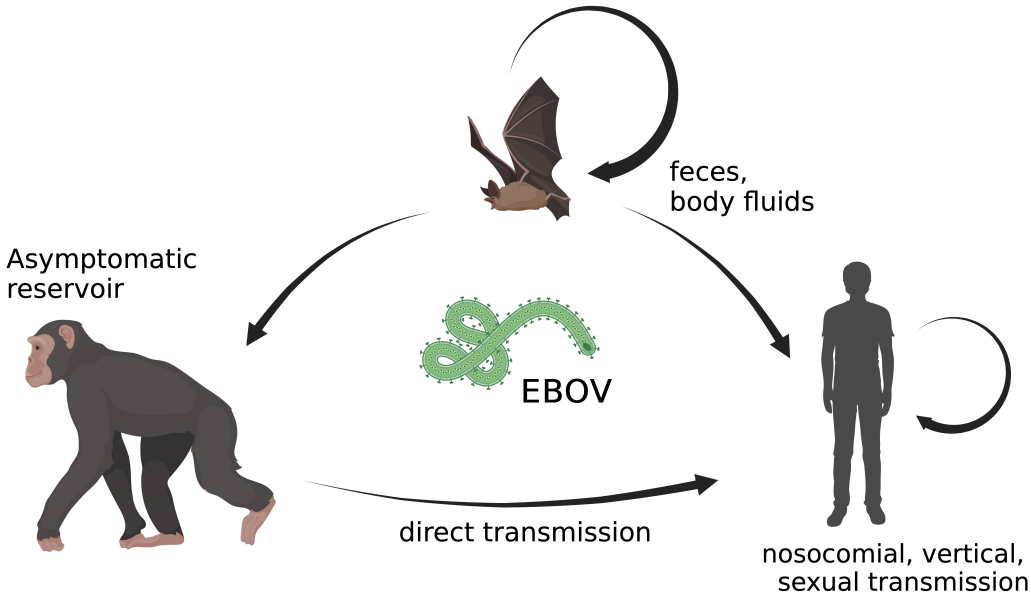


Figure 1.7: **Proposed EBOV transmission.** Created in https://BioRender.com. Although most infections in an EBOV outbreak occur through human-to-human contact, the origins are thought to occure from the wild. Reported infections occurred through contact with chimpanzees or bats. However, the primary reservoirs have still not been identified conclusively (Sundaram *et al.* 2025).

in two neighbouring countries of Uganda, the DRC and Sudan (Paessler & Walker, 2013). The largest ever reported EBOV outbreak was the 2013-2016 West African EBOV outbreak, which likely followed human exposure to bats in Gueckedou prefecture, Guinea, and then spread widely through human-to-human contact, eventually affecting more than 28,000 people (WHO Ebola Response Team, 2016).

The only cases ever reported of EBOV in Uganda occurred during the 2019 outbreak in DRC, when three patients were detected in the Kasese district of Uganda (Aceng *et al.* 2020). An intensive public health response, including extensive surveillance, vaccination of healthcare workers, establishment of a frontline field laboratory (Schuh *et al.* 2021), and enhanced infection prevention and control (IPC) measures by the Uganda Ministry of Health, prevented onward dissemination (Aceng *et al.* 2020).

Ebola disease outbreaks with related species are more common in Uganda (Figure 1.8), including the first BDBV outbreak in the eponymous district in Uganda in 2007 (Wamala *et al.* 2010), the largest outbreak today of SUDV in 2000 in Gulu district (CDC, 2001), and the most recent SUDV outbreak officially declared over in April 2025 (WHO, 2025b). All affected districts from 2000 to 2024, occurring either as the origin of an outbreak or with imported cases into the district, are presented in Figure 1.8.

1.3.3 Rift Valley fever virus (RVFV)

RVFV is in the family of *Phenuiviridae* and in the genus *Phlebovirus*. It falls in the same order and class as CCHFV, the order *Hareavirales* and the class *Bunyaviricetes* (Kuhn *et al.* 2024). The genome structure is similar within the order and is shown in Figure 1.3. The segmented RNA genome is bound to nucleoprotein, the viral RNA-dependent RNA-polymerase L, and a membrane containing Gn and Gc surface glycoproteins (Kimble *et al.* 2024).

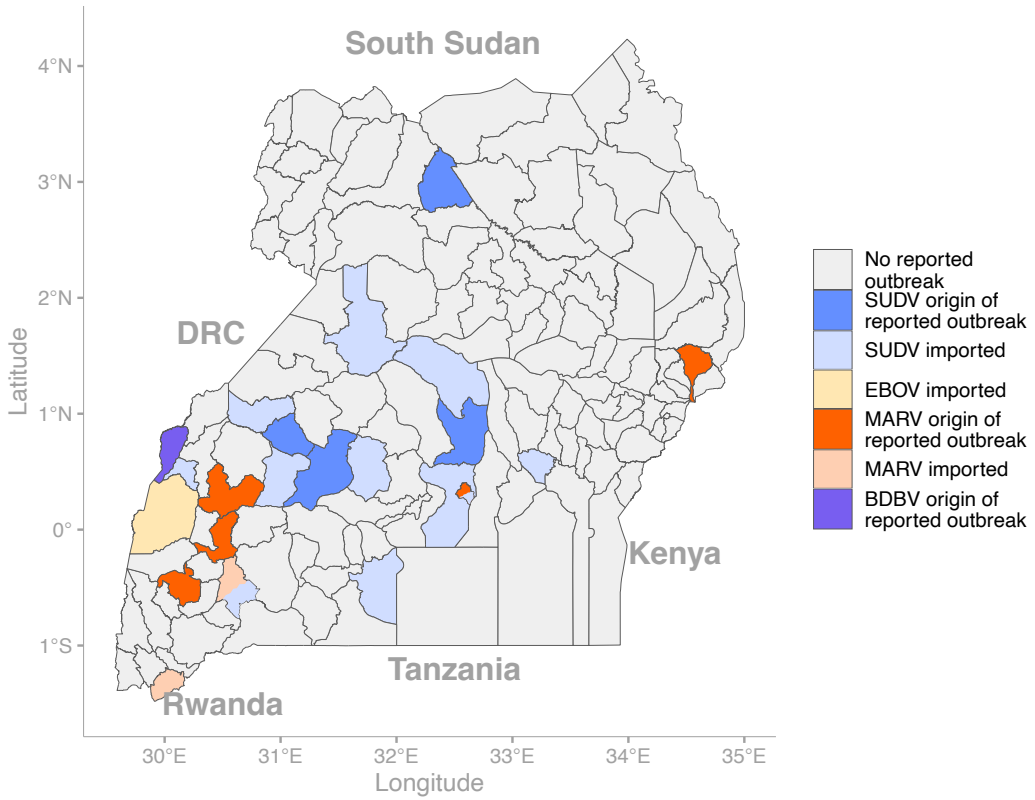


Figure 1.8: **Map of Uganda showing previous orthoebolavirus outbreaks.** Data on outbreaks available between 2000 and 2024, from (Balinandi *et al.* 2022) and personal communications. Districts of outbreaks are either defined as the origin where the outbreak started or as affected sites where the virus was imported from.

Distinct viral proteins of RVFV include the 78 kDa protein (important for mosquito infection), NSm (modulation of the host cell environment), and NSs (modulation of the host immune response).

Gn and Gc proteins are responsible for virus attachment and host cell entry. After uptake via caveolin-1-mediated endocytosis and fusion of the viral and endosomal membrane, replication and translation occur within the cytoplasm, similar to that described for CCHFV. Gn and Gc are translated at the rough ER and transported to the Golgi apparatus, where they form new virus particles by budding into the Golgi. Within the Golgi, virus particles are transported to the plasma membrane and released by exocytosis (Spiegel *et al.* 2016). Initial infection occurs within antigen-presenting cells, which carry the virus through draining lymph nodes to the liver and the spleen, which are the primary sites of replication (Kimble *et al.* 2024).

Disease susceptibility and severity vary with animal species and age. Foetal sheep and goats are highly susceptible, leading to a typical presentation with high abortion rates in infected animals (up to 80-100% (Bird *et al.* 2009)). In humans, the disease usually presents with a mild and self-limiting febrile illness. However, in some cases it can develop into severe disease, characterised by jaundice, rhinitis, encephalitis and haemorrhage (Anywaine *et al.* 2024; Bird *et al.* 2009; Shoemaker *et al.* 2019). In Uganda, a study by Anywaine *et al.* 2024 summarised case-fatality rates of RVFV, which, due to the bias in reporting and case handling, range widely between 0% and 53% (Anywaine *et al.* 2024; Nanyingi *et al.* 2015).

No specific treatment against RVFV is available for use in humans, and human cases are mainly managed with general supportive care (Afshar Moghaddam *et al.* 2025). Vaccines are available for use in livestock, but no human vaccine is currently licensed (Alkan *et al.* 2023; Kitandwe *et al.* 2022).

RVFV infections are predominantly diseases of domestic and wild ruminants,

transmitted by bites of infected mosquitoes (Smithburn *et al.* 1948) of the *Aedes*, *Anopheles* and *Culex* species (Seufi & Galal, 2010). In humans, RVFV is commonly acquired through contact with bodily fluids of infected domestic animals or through mosquitoes (Cecilia *et al.* 2022). A simplified transmission cycle is presented in Figure 1.9.

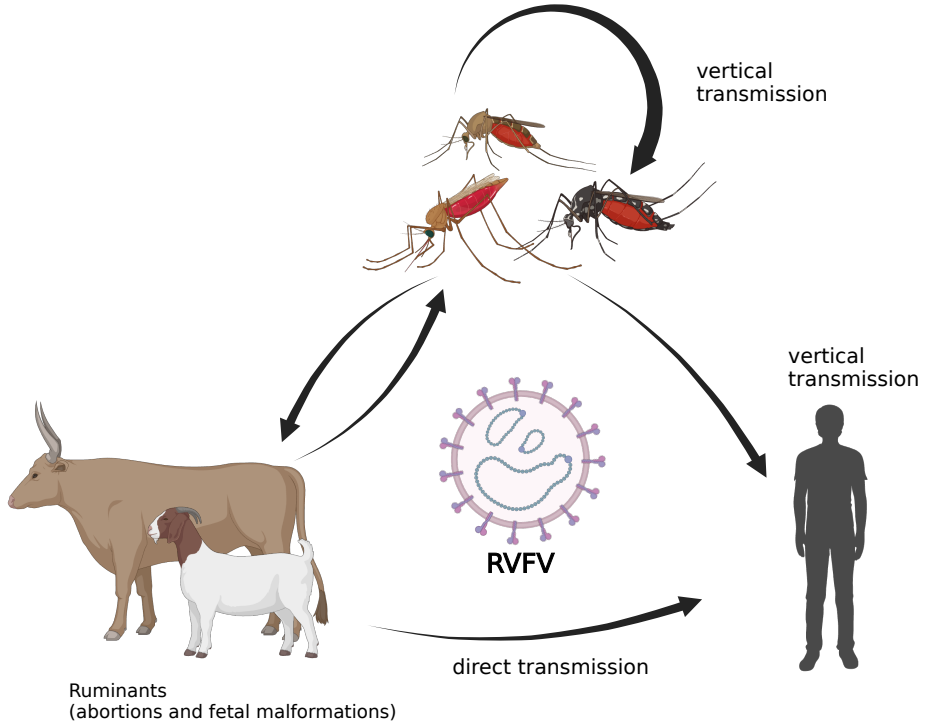


Figure 1.9: **Simplified RVFV transmission.** Created in https://BioRender.com. Predominantly, RVFV is transmitted by bites of infected mosquitoes to domestic and wild ruminants. Humans can be infected by mosquito bites, but are more often infected by contact with infectious animal blood or tissue. Within mosquito populations, the virus can be transmitted vertically.

The virus was first isolated during an outbreak on a sheep farm in Kenya in 1930 (Daubney *et al.* 1931), but has likely been present for many centuries, based on genomic data analysed in Bird *et al.* 2007. Most countries in Sub-Saharan Africa have reported RVFV outbreaks, as well as the Arabian Peninsula. With climate change and regular animal movements, the presence of RVFV is likely to spread further (Nair *et al.* 2023). In Uganda, the first

observation of RVFV was reported by Smithburn *et al.* 1948 from a field collection of mosquitoes in 1944. Human outbreaks have been reported since then (Shoemaker *et al.* 2019), including serological evidence of human and livestock infections (Nyakarahuka *et al.* 2018). Districts reporting human RVFV cases are presented in Figure 1.10.

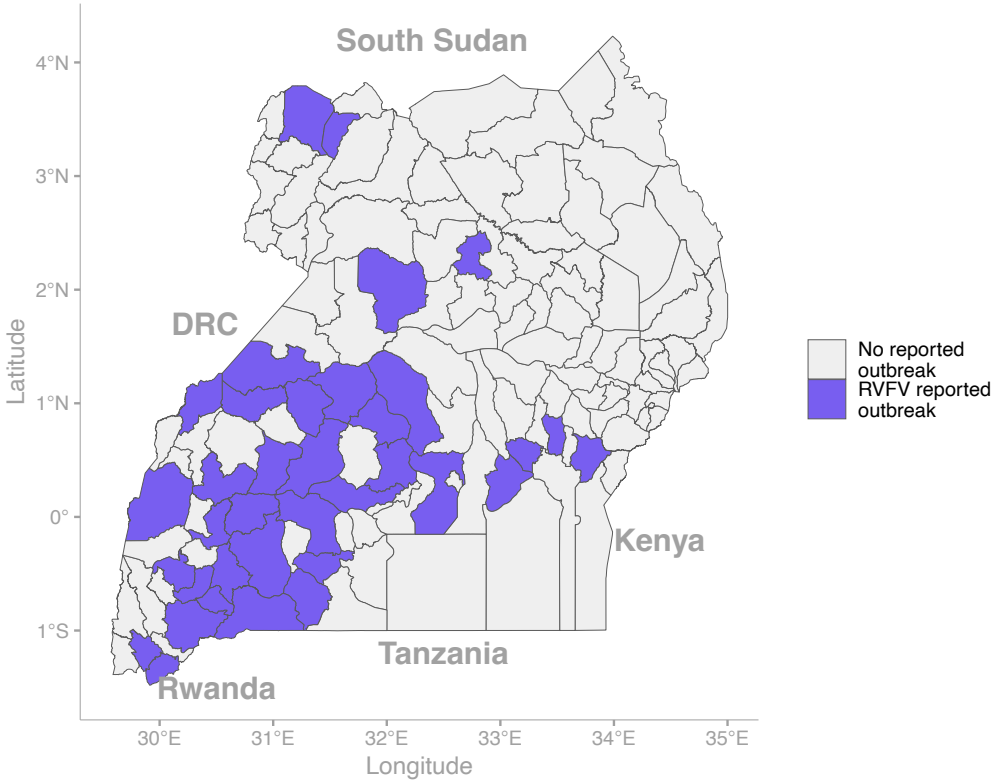


Figure 1.10: **Map of Uganda showing previous RVFV outbreaks.** Data on outbreaks between 2000 and 2024 from (Balinandi *et al.* 2022) and personal communication (Balinandi).

1.4 Research gaps

VHFVs such as CCHFV, EBOV, and RVFV are of growing concern to Uganda and the world, due to their potential for large outbreaks, high mortality, limited treatment options and complex transmission dynamics alongside geographical expansion due to climate change and land use changes.

Reports of outbreaks and cases of CCHFV in Uganda are increasing, challenging long-held assumptions that infections only occur sporadically as epidemics, are confined to the cattle corridor, and/or affect only farmers.

Research around human infections with VHFVs and specifically CCHFV focused mainly around outbreaks (including outbreak investigations and exposure in nearby communities (Atim, Niebel, *et al.* 2023; Balinandi *et al.* 2018, 2024)) and assumed high-risk groups such as abattoir workers and farmers (Atim *et al.* 2022; Lule *et al.* 2022).

Human exposure data from the general population is missing (Lule *et al.* 2022; Switkes *et al.* 2016), which can be used to study exposure risk, model transmission, and identify high-risk areas in Uganda. Additionally, understanding local contexts in risk behaviours is mostly overlooked. Mixed methods can help to get a wider insight into the current situation of CCHFV in Uganda, and guide intervention strategies.

1.5 Research aims and hypotheses

This research aimed to gain a deeper understanding of mechanisms of human exposure to VHFVs in Uganda and is divided into three main aims:

- **1.** To investigate risk factors associated with exposure to EBOV, RVFV and CCHFV in healthcare workers (HCWs) compared to local communities, using data from a cross-sectional serosurvey from five study sites in Uganda.
- **2.** To understand local differences associated with human-animal-tick interactions in six environmental and socioecologically distinct districts of Uganda, by conducting focus group discussions (FGDs) and key informant interviews (KIIs).

- **3.** To estimate exposure to CCHFV in four districts and analyse associated risk behaviours using a cross-sectional, household-based, randomised serosurvey.

The following hypotheses were tested and discussed within this research:

- **1.** HCWs in Uganda are at significantly higher risk of exposure to VHFVs, mainly driven by occupational contact patterns, compared to members of the general community.
- **2.** Socioecological behaviours related to human-animal and human-tick interactions vary across Uganda and may indicate potential risk behaviours for exposure to CCHFV.
- **3.** Geographic heterogeneity in CCHFV seroprevalence across Uganda is partially attributed to socioecological behaviours.

1.6 Thesis outline

In this thesis, I used a mixed methods approach to investigate and understand the complex connections of exposure risks to VHFVs, concentrating mostly on CCHFV in Uganda. To address the study aims, the mixed methods approach comprises quantitative and qualitative components (Östlund *et al.* 2011). These components each have strengths and challenges, and using them in combination in this study was carried out to address the research questions.

Quantitative studies typically include large and representative samples, leading to generalisable and objective results. However, in quantitative studies, it is important to ask the right questions for the results to be meaningful and complete. Additionally, they can lack depth and can overly simplify complex challenges. Such simplification may cause problems when the results are

used to address and suggest health interventions, without a detailed knowledge of the behaviours or the reasoning behind them. To address this, we also conducted a qualitative study, which later informed the study design, the discussion and the conclusions of the quantitative study.

The thesis outline and the chapters' relations to each other are pictured in Figure 1.11. Chapter 2 presents a cross-sectional occupational study among healthcare workers in Western and Northern Uganda. Healthcare workers were recruited from five hospitals in three districts of Uganda, and sex and age matched with surrounding community members. I present demographic characteristics, seroprevalences for EBOV, CCHFV, and RVFV, and multivariable logistic regression analyses to identify risk factors.

The strong dependence of seropositivity for CCHFV by study site led us to focus further work on CCHFV to investigate this phenomenon more deeply. Chapter 3 is the first of three chapters (Chapter 3 to 5) that are closely interrelated. In Chapter 3, I explain the method and the results of selecting distinct study sites in Uganda. Both studies described in Chapter 4 and Chapter 5 were conducted in a sub-selection of these sites.

In Chapter 4, I present a qualitative study investigating human-animal-tick interactions across Uganda. We travelled to all six study sites selected in Chapter 3, and conducted FGDs and KIIs with community members. Results on behaviours regarding animals and ticks are presented alongside quotes and discussed in the chapter. This qualitative study informed the quantitative research in Chapter 5.

We carried out a quantitative study within the same study sites focused on the seroprevalence and risk of exposure to CCHFV in Uganda. Participants were recruited within districts, randomised for village, household and individual participant levels. I present interim results from the first four districts, including demographics, estimated seroprevalence for districts and villages, and

multivariable logistic regression analyses to identify risk factors.

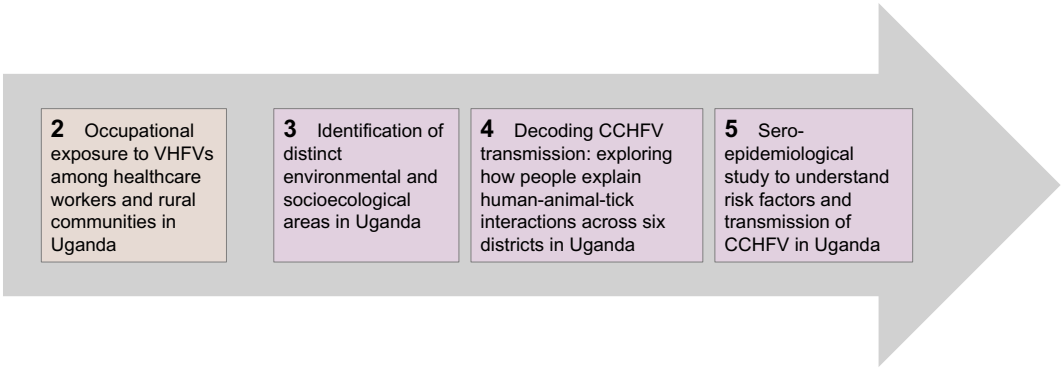


Figure 1.11: **Thesis outline.** All chapters follow chronologically and logically after each other. Chapter 3 to Chapter 5 are strongly interlinked

Finally, during my thesis, as a community engagement project, I developed a board game, called Vector Ludo, based on the popularity of Ludo in Uganda, in order to engage and inform community members about vector-borne diseases in Uganda. The process and the final board are presented in Chapter 6.

Lastly, Chapter 7 presents the overall findings and discusses their significance in the wider context. I then consider the limitations of the results presented in the thesis and provide suggestions for future research.

Chapter 2

Occupational exposure to viral haemorrhagic fever viruses among healthcare workers and rural communities in Uganda

2.1 Abstract

Outbreaks of viral haemorrhagic fevers (VHFs) are common in Uganda, where healthcare workers (HCW) and local communities are at high risk of exposure. We aimed to identify risk factors associated with exposure to (Ebola virus (EBOV)), (Crimean-Congo haemorrhagic fever virus (CCHFV)) and Rift Valley fever virus (RVFV).

A case-control study was conducted among 639 healthcare workers (HCWs) and 714 age- and sex-matched community members from four high-risk study sites in Uganda. Blood was tested for EBOV and CCHFV seropositivity by enzyme-linked immunoassay and for RVFV by indirect immunofluorescence. Exposure risk factors were evaluated with a structured survey and analysed

by multivariable logistic regression.

Overall seropositivity was 16% for EBOV, 19% for CCHFV, and 2% for RVFV. The highest odds of exposure were noted in Arua district for both EBOV (AOR = 9.01 [95% CI = 5.48-15.4]) and CCHFV (AOR = 4.67 [95% CI = 3.11-7.13]), an area that has had no documented cases of VHFVs. Overall, HCWs had lower odds of EBOV exposure than community members (AOR = 0.37 [95% CI0.26-0.51]), as well as of CCHFV exposure (AOR = 0.42 [95% CI 0.31-0.57]). Homemakers and cleaners were the two occupational groups with the highest seropositivity for EBOV and CCHFV.

Our results underscore the importance of educating community members and HCWs in the identification of cases and prevention of transmission in hospitals and for carers, and the development of vaccines for use in outbreaks.

2.2 Acknowledgements

This chapter was submitted to The Lancet Global Health in May 2025 and is under review (as of September 2025). A complete author list of the submitted paper is shown below:

Kugler M, Burkert S, Ocen D L, Ashraf S, Atim S A, Shepherd J G, Blunsum A E, Davis C, Brady C, Tipton T, Kiggundu G, Mubiru A, Nassuna A C, Balinandi S, Kizito D, Omona V, Ocaka R, Kabugho L, Masereka E, Muhindo J, Musoki M, Munyagwa M, Atolere E, Muhindo R R, Harold O E, Apangu P, Aliotratra R, Aniku W, Dradiku C A, Amandu C H, Achanit F, Newton M, Denis A, Atiku L A, Downing R, Bwogi J, Johnson P C D, Caroll M, Houlihan C F, and Thomson E C.

A report, in lay language and reduced data complexity, has also been published on the open platform Enlighten by the University of Glasgow (https://doi.

org/10.36399/gla.pubs.349489). It was also shared with district officials in the study districts to provide feedback on the results in a summarised version.

This study involved many groups and people. Complete acknowledgement is presented in Table 2.1.

This study was supported by the UK Public Health Rapid Support Team (UK-PHRST). UK-PHRST is funded by UK aid from the Department of Health and Social Care and is jointly run by Public Health England and the London School of Hygiene & Tropical Medicine. The views expressed in this publication are those of the authors and not necessarily those of the National Health Service, the National Institute for Health Research or the Department of Health and Social Care. Additional funding came from the UK Medical Research Council (MRC) and the UK Foreign, Commonwealth & Development Office (FCDO) under the MRC/FCDO Concordat agreement and is carried out in the frame of the Global Health EDCTP3 Joint Undertaking (Preparedness Platform MC_UU_00034/6).

	Contributions
Marina Kugler	Leading recruitment in Kasese district, including training, creating documentation, overseeing finances RVFV assay in Glasgow Overseeing all labwork Data analysis, creating figures and tables
Sanne Burkert, Daniel Lukwiya Ocen, James G Shepherd, Stella A Atim, Andrew E Blunsum, Shirin Ashraf, Paul C D Johnson, Catherine Houlihan, Emma C Thomson	Initial setup and planning before the start of recruitment
Dr Venice Omona, Robert Ocaka, Susan Apiyo, Tiberius Odokonyero, Denis Okello, Jude Kwotek, Samuel Acire	Recruitment in Gulu district
Obitre Eyoa Harold, Pontius Apangu, Aliotratra Robinson, William Aniku, Christopher A Dradiku, Christopher H Amandu, Achanti Florence, Munakenya Newton, Agaba Denis, Linda A Atiku	Recruitment in Arua district
Laheri Kabugho, Edson Masereka, Joshua Muhindo, Esther Atolere, Robert Muhindo, Bettress Happy, Merecy Musoki, Mary Munyagwa	Recruitment in Kasese district
Caolann Brady, Tom Tipton, Miles Caroll	EBOV assay
Gladys Kiggundu, Dennison Kizito	CCHFV assay
Andrew Mubiru, Charity Nassuna Angela, Stephen Balinandi	RVFV assay at UVRI

Table 2.1: Contributions to HCW study.

2.3 Introduction

Spread of infection during viral haemorrhagic fever virus (VHFV) outbreaks occurs through direct contact with infected patients, putting healthcare workers and close community contacts at high risk. An increased risk of infection in HCWs was reported during the 2013-2016 West Africa Ebola virus outbreak (WHO Ebola Response Team, 2014), as well as during the Ebola disease outbreak in Gulu, Uganda, in 2006 (Okware *et al.* 2002). Nosocomial infections with CCHFV have been reported in several countries (Tsergouli *et al.* 2020), though person-to-person transmission of RVFV has not been recorded. Although using personal protective equipment (PPE) can prevent nosocomial infection, this is often only used during recognised outbreaks.

This chapter aimed to investigate risk factors associated with exposure to EBOV, RVFV and CCHFV in HCWs compared to local communities, using data from a cross-sectional serosurvey from five study sites in Uganda. The following objectives are addressed:

- **1.1** To analyse seroprevalence of EBOV, RVFV and CCHFV in HCWs compared to local communities.
- **1.2** To identify and analyse risk factors associated with EBOV, RVFV and CCHFV exposure in HCWs and local communities, using univariable and multivariable logistic regression models.
- **1.3** To identify and analyse occupational risk groups associated with EBOV, RVFV and CCHFV exposure in HCWs and local communities, using univariable and multivariable logistic regression models.

2.4 Methods

Ethics

The AVI study was approved by the UVRI REC (GC/127/18/09/662; Appendix B, p.210) and by the Uganda National Council for Science and Technology (UNCST; HS 2485; Appendix B, p.215). Written informed consent was obtained from all study participants.

Study design

Sites were identified in northern (Arua Regional Referral Hospital and Adumi Health Centre IV; January-March 2019) and western regions (Lacor Hospital, Gulu; August-September 2019 and Kagando Hospital/ Bwera Hospital; December 2020- January 2021). Sites and historical VHFVs outbreak locations up to 2024 are shown in Figure 2.1, Figure 1.5 (CCHFV), Figure 1.8 (EBOV), and Figure 1.10 (RVFV). All hospitals had recorded cases of VHF outbreaks at some time in the past, except for the healthcare facilities in Arua district.

Selection of study participants

Individuals working in hospitals in any role were categorised as HCWs. We aimed for a ratio of 1:1.5 of HCWs to community members for 80% power to detect an odds ratio of 2.2 or greater, assuming a VHFV seroprevalence of 6% or lower in the community. 150 HCWs were included from each hospital, and age- and sex-matched with up to 225 local community members, recruited from within 5km of hospital sites. Study recruitment details are shown in Figure 2.2.

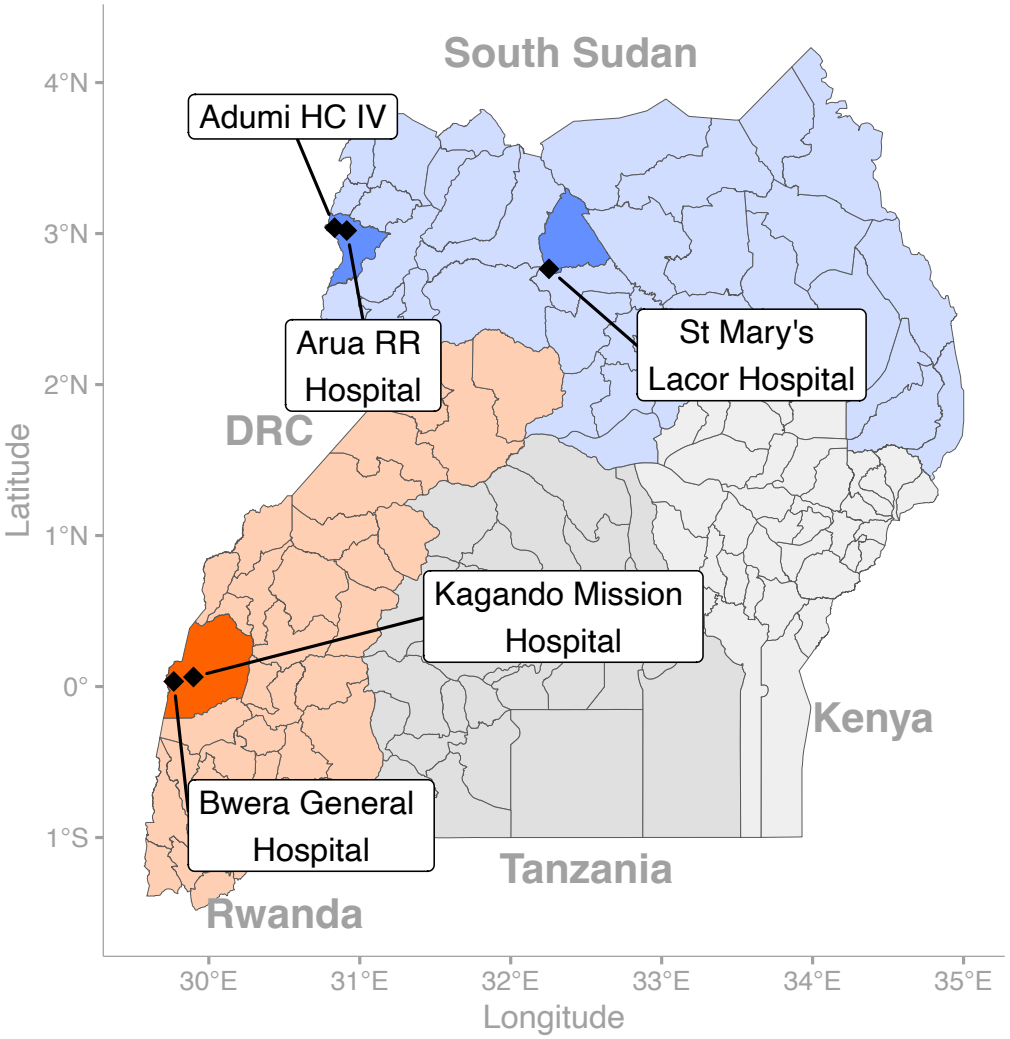


Figure 2.1: **Map of Uganda showing study sites.** Sampled districts are shown in dark blue and dark orange, and the respective regions in light blue (Northern region) and light orange (Western region).

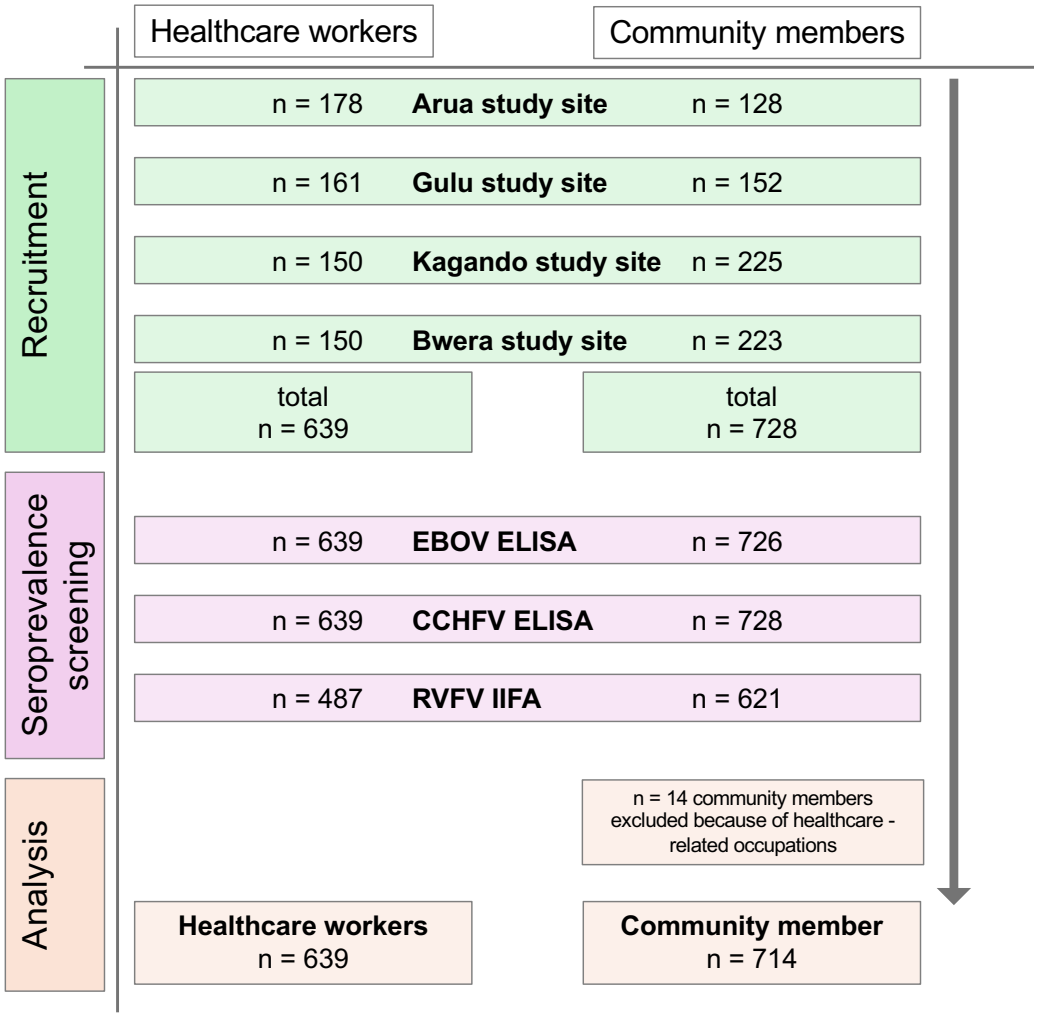


Figure 2.2: **Flowchart of study.** Healthcare workers are shown on the left side, with their control group of community members on the right side. Separated by recruitment, seroprevalence screening and analysis.

Data and sample collection

Sociodemographic characteristics and risk factors for VHFV exposure were recorded using a structured questionnaire in local languages. A 10ml venous blood sample was collected, centrifuged at 2000g for 10min, and aliquoted into 2ml sterile storage vials (Sarstedt Inc, Newton, North Carolina). Serum was heat-inactivated at 56°C for 30min, stored short-term at –20°C and then at –80°C.

Serological assays

Serum samples were tested with IgG enzyme-linked immunosorbent assays (ELISA) using EBOV glycoprotein (GP) and nucleoprotein (NP). The EBOV GP assay has been described previously (C. Davis *et al.* 2020; Thom *et al.* 2021). Briefly, plates were coated with 1µg/mL recombinant EBOV GP (recombinant, produced in HEK293 cells with a C-term His-tag, Native Antigen Company, UK). After blocking with casein for 1 hour at room temperature, sera were added in triplicate at two dilutions (1:100 and 1:500). Secondary anti-IgG, alkaline phosphatase conjugated, was added at a concentration of 1:1000. The plates were incubated for 20 minutes with Diethanolamine and p-Nitrophenyl Phosphate. Optical density was determined at 405nm. A graphical description is shown in Figure 2.3. The EBOV NP assay substituted NP antigen (recombinant, expressed and purified from *E. coli*, Native Antigen Company, UK) at a concentration of 0.5µg/mL. The rest of the protocol was kept exactly the same. The antigen concentration was determined by Caolann Brady and Tom Tipton through checkerboard titrations of the NP antigen in three convalescent Ebola virus disease patients. The pool of the three convalescent samples had a combined anti-NP titre of 6IU/ml, quantified against WHO controls. Convalescent samples were added to each plate as positive controls, as well as a pool of negative control samples. Since vaccination with

rVSV-ZEBOV (a GP-based vaccine) had been previously rolled out in two districts (Gulu and Kasese), the NP assay was used for the comparative analysis to avoid bias from vaccination.

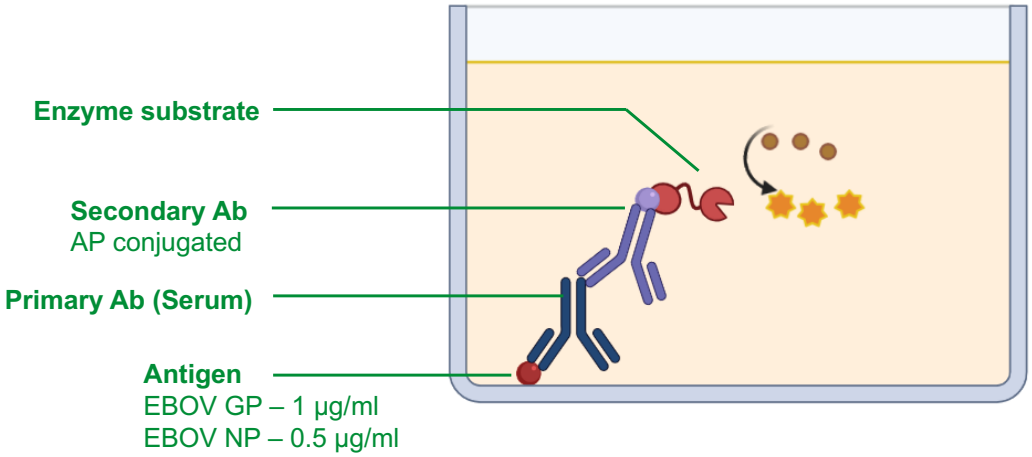


Figure 2.3: **Ebola virus ELISA for GP and NP.** Graphical representation of ELISAs, highlighting coating with antigen (GP or NP), primary antibody when present in sample serum, secondary antibody conjugated with alkaline phosphatase (AP), and enzyme substrate (Diethanolamine and p-Nitrophenyl Phosphate). Figure adapted from Caolann Brady.

Sera were tested for CCHFV IgG using the Vector Best IgG ELISA (Novosibirsk, Russia) following manufacturers' instructions. Briefly, sample serum was added to coated wells (whole virus antigen of unknown origin), at a final dilution of 1:100. Positive and negative controls were supplied by the kit and added on each plate. After incubation for 1 hour at 37°C the plates were washed. The antibody conjugate (anti-IgG with conjugated Horseradish Peroxidase (HRP)) was added and incubated for 30 minutes at 37°C. After washing, the substrate solution, consisting of Tetramethylbenzidine (TMB), was added and incubated at room temperature and in the dark for 25 minutes. The provided stop solution is added, and optical density is measured at 450nm. Result interpretation was conducted using the controls. An equivocal interpreted result led to repeated testing, and was considered negative after a second equivocal result.

An indirect immunofluorescence assay (Euroimmun, Lübeck, Germany) was used to test for RVFV IgG, following the manufacturer's instructions. In short, infected and uninfected cells were immobilised on a biochip supplied with the kit. 1:100 diluted sample serum was added and incubated for 30 minutes. After washing, fluorescein-labelled anti-human IgG was added. After the final wash, a mounting medium and a cover glass were added. Fluorescence microscopy was used to evaluate seroprevalence in serum samples. An example of a positive and a negative sample is shown in Figure 2.4, highlighting the specific patterns of infected cells when the antibody is bound.

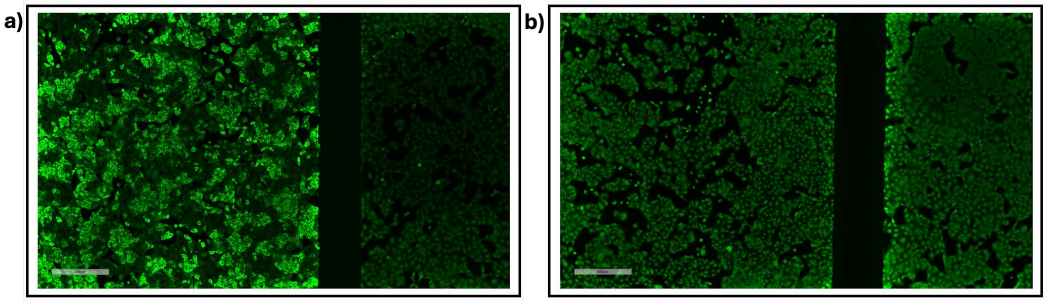


Figure 2.4: **Indirect immunofluorescence assay** (Euroimmun, Lübeck, Germany) (a) positive example microscope picture with typical antibody binding to infected cells on the left side compared to the right side as internal control of uninfected cells (b) negative example microscope picture

Statistical analysis

Data were analysed using R 4.2.0 (R Core Team, 2021) and maps and graphs created with ggplot2 (Hadley Wickham, 2016). HCWs recruited as community members were excluded from the analysis. Ages are presented in ten-year age brackets. Univariable and multivariable logistic regression analysis were performed separately for EBOV, CCHFV, RVFV using the glm function and the gtsummary package (R Core Team, 2021; Sjoberg *et al.* 2021). Initial risk analysis was performed for known risk factors, including exposure to wild animals, caves and bats for EBOV, animal slaughtering and exposure to ticks

for CCHFV, and animal slaughtering, death of own animal and exposure to mosquitoes for RVFV. This was followed by an exploratory analysis incorporating all risk variables recorded using a stepwise analysis approach with a cutoff of $p < 0.2$. To investigate at-risk occupations, we conducted univariable and multivariable logistic regressions within occupational cadres for HCWs and community members. Collinearity in all models was examined by calculating variance inflation factors (VIFs) using the car library (Fox John & Weisberg Sanford, 2019). Variables that attained a p-value of < 0.05 were considered to be statistically significant.

2.5 Results

Baseline characteristics of the study population

A total of 1,353 participants (639 healthcare workers and 714 community members) were enrolled from two sites (Arua district and Gulu district) in the Northern region (618/1,353; 45.7%) and two sites (Kagando and Bwera both in Kasese district) in the Western (735/1,353; 54.3%) region (Table 2.2; Figure 2.1). There were more females (901/1,353; 66.6%) than males (452/1,353; 33.4%). The median age was 32 years (IQR = 24, 42), ranging from 18 to 77 years. A total of 183 participants (15.4%) reported being vaccinated with the rVSV-ZEBOV-GP vaccine, of which the majority were healthcare workers (181/183; 98,9%).

Nurses formed the largest group of any single occupation within hospitals (270/639; 42.3%), followed by cleaners (102/639; 16.0%), midwives (85/639; 13.3%), doctors (52/639; 8.1%) and laboratory personnel (32/639; 5.0%) (Figure 2.5a). These categories comprised more females than males, accounting for the female preponderance in the study. In the community study group, the largest single occupation was farming and animal handling (213/714; 29.8%),

	Healthcare worker (N=639)	Community member (N=714)	Total (N=1353)
Study location			
Kasese district (Bwera)	150 (23.5%)	222 (31.1%)	372 (27.5%)
Kasese district (Kagando)	150 (23.5%)	213 (29.8%)	363 (26.8%)
Arua district	178 (27.9%)	128 (17.9%)	306 (22.6%)
Gulu district	161 (25.2%)	151 (21.1%)	312 (23.1%)
Sex			
Female	416 (65.1%)	485 (67.9%)	901 (66.6%)
Male	223 (34.9%)	229 (32.1%)	452 (33.4%)
Age (years)			
18 to 27	204 (31.9%)	304 (42.6%)	508 (37.5%)
28 to 37	170 (26.6%)	200 (28.0%)	370 (27.3%)
38 to 47	154 (24.1%)	116 (16.2%)	270 (20.0%)
48 to 57	89 (13.9%)	66 (9.2%)	155 (11.5%)
58 to 77	22 (3.4%)	28 (3.9%)	50 (3.7%)
rVSV-ZEBOV-GP vaccine			
Yes	181 (28.5%)	2 (0.4%)	183 (15.4%)
No	453 (71.5%)	555 (99.6%)	1008 (84.6%)

Table 2.2: **Demographic data of participants by study group.** Excluding unknown or blank data. The full dataset is available in Table A.1.

followed by business (157/714; 22.0%), casual work (93/714; 13.0%), homemaker (87/714; 12.2%), and education (80/714; 11.2%) (Figure 2.5b).

EBOV seroprevalence

Seropositivity for EBOV NP was detected in 221 of 1,131 (16.3%) participant samples. There was no significant difference in seropositivity for EBOV NP between participants who reported being vaccinated (24/183; 13.1%) and unvaccinated (167/1,007; 16.6%) ($p = 0.286$). As expected, EBOV GP seroprevalence was higher in the vaccinated group; 67.8% (124/183) versus 11.5% (116/1,007; $p < 0.001$) in the unvaccinated group (Figure 2.7). Correlation analysis demonstrated a weak but statistically significant positive association between EBOV GP and EBOV NP specific antibody responses (Pearson's $R = 0.24$, $p = 2.1 \times 10^{-13}$), confirming our decision to use EBOV NP as a

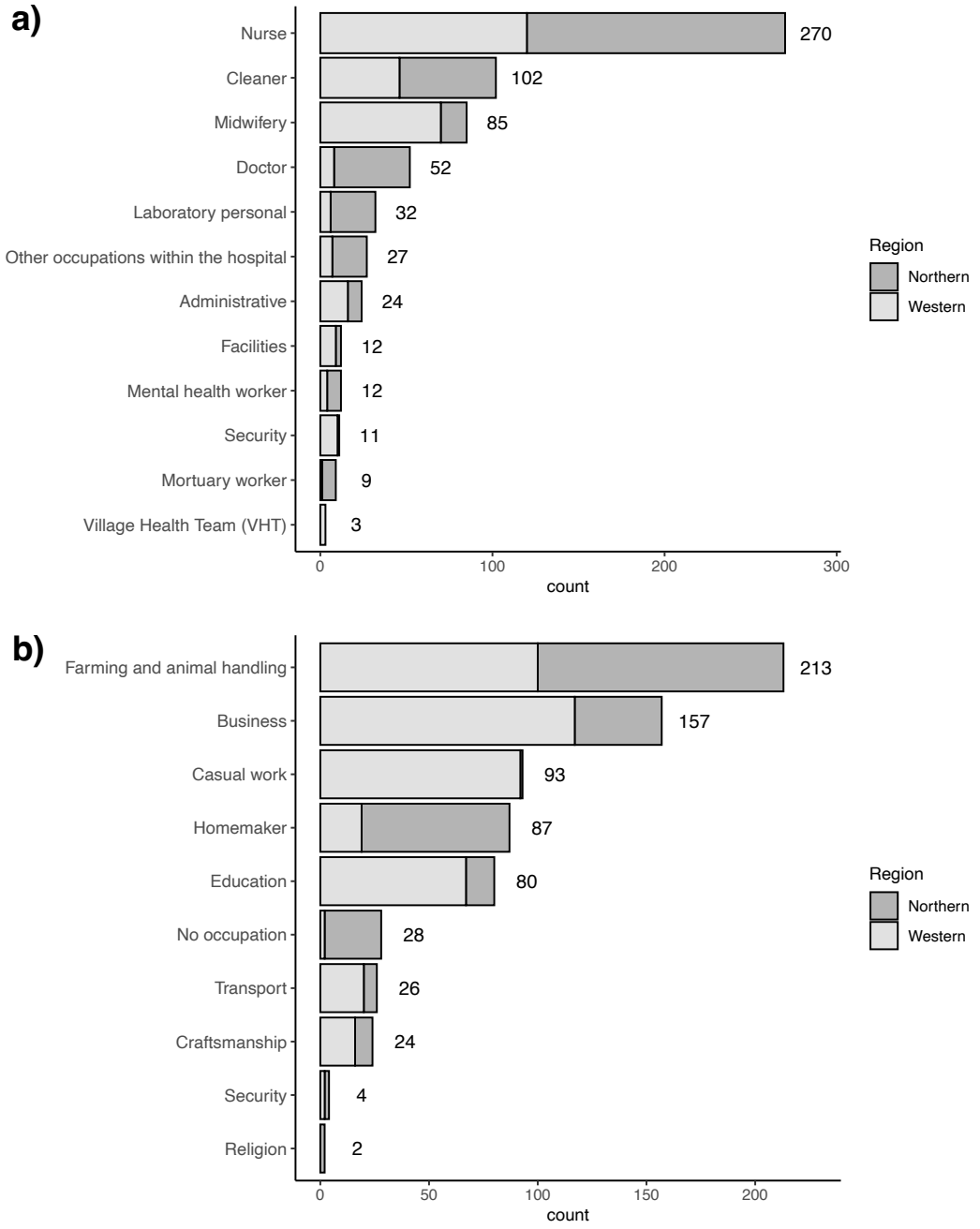


Figure 2.5: **Occupational cadres separated by region** for (a) HCWs and (b) Community members. The colour of the bars represents the region: dark grey (Northern Region) and light grey (Western Region). The x-axis represents the total counts in each occupation cadre.

measurement of seropositivity (Figure 2.6).

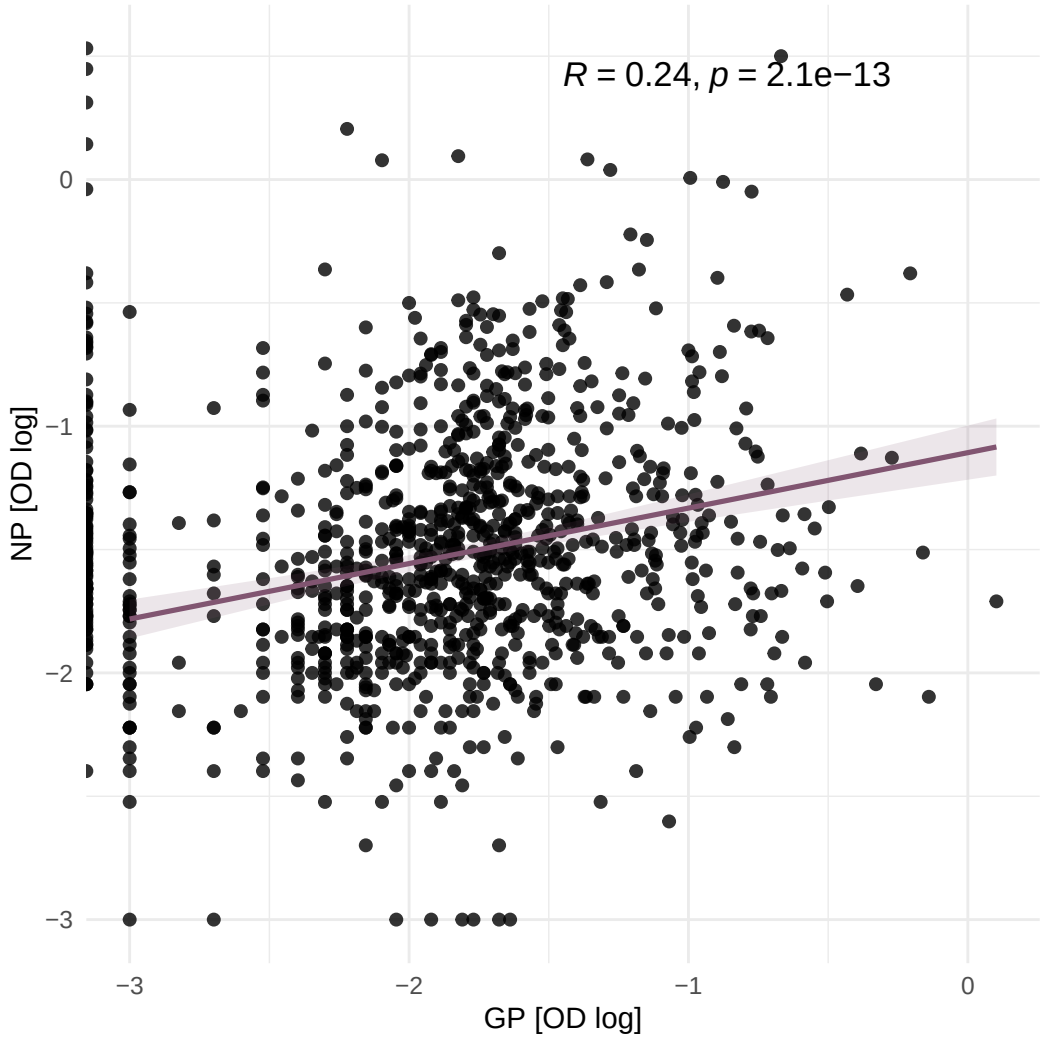
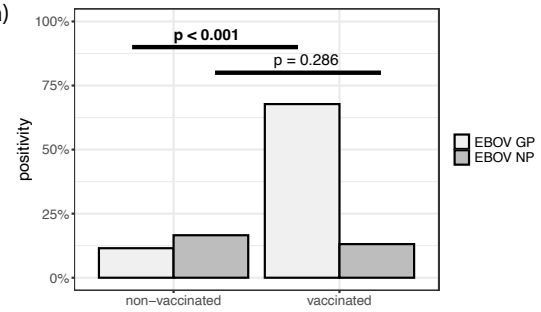


Figure 2.6: **Correlation plot for ELISA results for EBOV GP and NP.** Pearson's correlation is presented in the plot, with OD values for EBOV GP on the x-axis and EBOV NP on the y-axis.

A higher proportion of individuals was positive for EBOV NP IgG in the Northern region (153/618; 24.8%), with the highest seropositivity in Arua district (96/306; 31.4%) compared with Gulu district (57/312; 18.3%) ($p < 0.001$) (Figure 2.8a-b). In the West, a higher seroprevalence was observed in Kagando hospital (43/362; 11.9%), versus Bwera hospital (25/372; 6.7%) ($p = 0.022$) (Figure 2.8c).

Overall, HCWs had a lower EBOV NP seroprevalence (77/639; 12.1%) com-

a)



b)

	GP-	GP+	sum
NP-	750	90	840
NP+	141	26	167
sum	891	116	

c)

	GP-	GP+	sum
NP-	53	106	159
NP+	6	18	24
sum	59	124	

Figure 2.7: **ELISA results for EBOV GP and NP.** (a) Seropositivity for EBOV GP and EBOV NP in participants who reported being vaccinated for EBOV versus those who reported that they were not vaccinated. (b) 2 by 2 table showing EBOV GP seroprevalence results against EBOV NP seroprevalence results in reported non-vaccinated, and (c) in reported vaccinated participants.

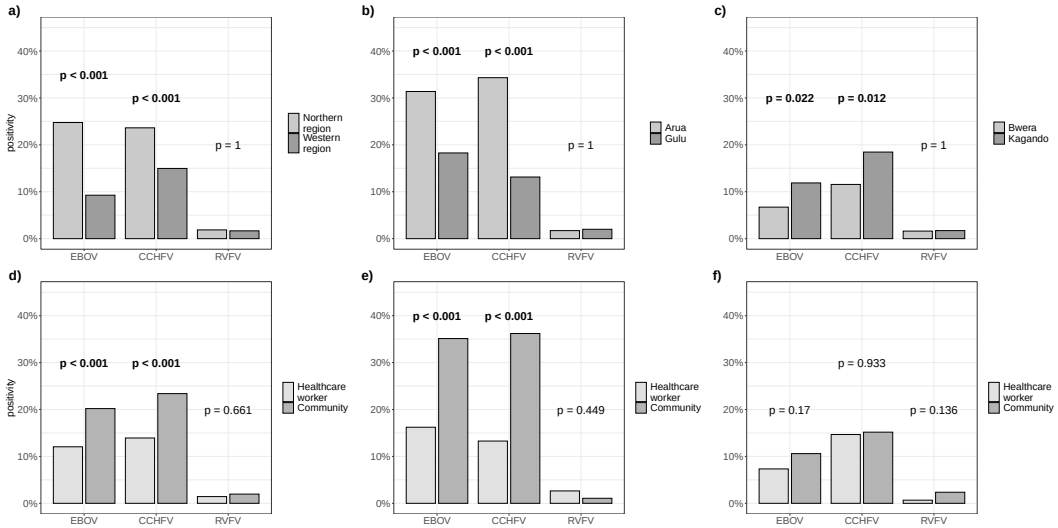


Figure 2.8: **VHFV seropositivity in healthcare workers and community members in Uganda.** The x-axis shows the type of VHFV tested (Ebola virus (EBOV) NP seroprevalence, Crimean-Congo haemorrhagic fever virus (CCHFV) tested with the VectorBest assay, and Rift Valley fever virus (RVFV)). The y-axis shows percentage seropositivity. (a) Data for both study groups by geographical area – Northern (light grey) versus Western (grey) Regions. (b) Northern region only – Arua district (light grey) vs Gulu district (grey). (c) Western region only – Bwera hospital (light grey) versus Kagando hospital (dark grey) (d) Data for both regions by study group - HCWs (light grey) vs community members (dark grey) (e) Seroprevalence by occupation in Northern Region (f) Seroprevalence by occupation in Western Region.

pared with community members (144/713; 20.2%) ($p < 0.001$). This trend was driven by differences in the Northern region (HCWs: 55/339; 16.2% versus community members: 98/279; 35.1%; $p < 0.001$) rather than the Western region (HCWs: 22/300; 7.3% vs community members: 46/434; 10.6%; $p = 0.170$) (Figure 2.8d-f). The viral haemorrhagic fever viruses (VHFVs) seroprevalence by occupational group is shown in Table A.2. The highest percentage for EBOV seropositivity was detected in cleaners (23/102; 22.5%) within the HCWs group, and homemakers (39/87; 44.8%) within the community member group (Table 2.3).

Risk factors for EBOV seropositivity

In the multivariable regression model (Figure 2.9a, Table A.3), there was an association with study location (Arua district: AOR = 9.01; 95% CI = 5.48-15.4; Gulu district: AOR = 4.15; 95% CI = 2.43-7.31; Kagando in Kasese district: AOR = 2.19; 95% CI = 1.27-3.86; Bwera in Kasese district as reference; $p < 0.001$). Being a HCW was associated with a lower odds of EBOV seropositivity by NP assay (AOR = 0.37; 95% CI = 0.26-0.51 $p < 0.001$). Male sex was associated with higher odds of seropositivity (AOR = 1.57; 95% CI = 1.13-2.17; $p = 0.008$).

Two additional analyses were conducted to investigate differences in occupational cadres within the study groups. Multivariable regression analysis with correction for study location, sex and age, demonstrated a significant difference in the odds of being EBOV seropositive between HCW occupations (Table 2.3a). The highest odds occurred in cleaners (AOR = 3.39; 95% CI = 1.70-6.79; $p = 0.002$). A multivariable regression in community members showed no significance by occupation, although this was significant in the univariable analysis (Table 2.3b).

In an exploratory multivariable regression analysis (Table A.4), a similar pat-

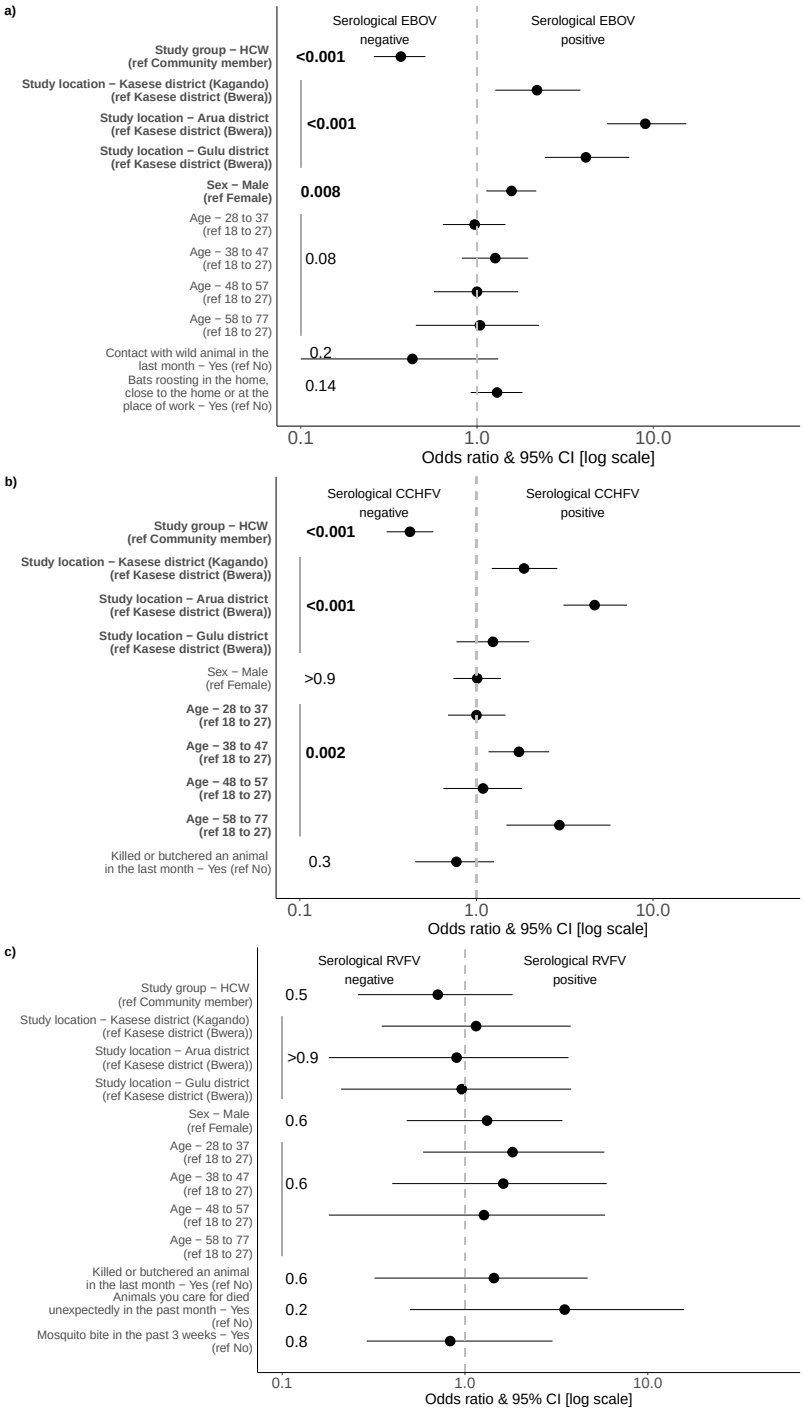


Figure 2.9: **Risk factor analysis for seropositivity risk to VHFVs in multivariable logistic regression analyses** (a) EBOV NP seroprevalence (b) CCHFV seroprevalence tested with VectorBest assay (c) RVFV. Data on the x-axis shows the odds ratio and 95% confidence interval for the risk factors listed on the y-axis. P-values are shown for individual or groups of variables to the left of the data points. A dotted line runs vertically on each graph to indicate a neutral risk of OR = 1.

a)

Characteristic	EBOV positive, N = 68[1]	EBOV negative, N = 473[1]	Univariable regression			Multivariable regression		
			OR[2]	95% CI	Univariable p.value	AOR[3]	95% CI	Multivariable p.value
Study location					**0.002**			**<0.001**
Kasese district (Bwera)	11 (9.6%)	103 (90.4%)	—	—		—	—	
Kasese district (Kagando)	7 (5.1%)	129 (94.9%)	0.51	0.18, 1.34		0.42	0.15, 1.14	
Arua district	23 (16.4%)	117 (83.6%)	1.84	0.87, 4.10		1.66	0.74, 3.91	
Gulu district	27 (17.9%)	124 (82.1%)	2.04	0.99, 4.47		2.66	1.18, 6.37	
Sex					0.14			**0.017**
Male	27 (15.7%)	145 (84.3%)	1.49	0.87, 2.50		2.24	1.16, 4.34	
Female	41 (11.1%)	328 (88.9%)	—	—		—	—	
Age					0.5			0.7
18 to 27	18 (9.7%)	167 (90.3%)	—	—		—	—	
28 to 37	18 (13.1%)	119 (86.9%)	1.40	0.70, 2.82		0.96	0.44, 2.09	
38 to 47	22 (16.5%)	111 (83.5%)	1.84	0.94, 3.62		1.00	0.47, 2.15	
48 to 57	8 (11.3%)	63 (88.7%)	1.18	0.46, 2.77		0.63	0.23, 1.63	
58 to 77	2 (13.3%)	13 (86.7%)	1.43	0.21, 5.73		0.43	0.06, 1.96	
Occupation					**0.007**			**0.002**
Nurse	29 (10.7%)	241 (89.3%)	—	—		—	—	
Cleaner	23 (22.5%)	79 (77.5%)	2.42	1.31, 4.42		3.39	1.70, 6.79	
Doctor	5 (9.6%)	47 (90.4%)	0.88	0.29, 2.22		0.49	0.15, 1.36	
Laboratory personnel	6 (18.8%)	26 (81.3%)	1.92	0.67, 4.79		1.28	0.41, 3.57	
Midwifery	5 (5.9%)	80 (94.1%)	0.52	0.17, 1.28		1.15	0.35, 3.31	

[1] n (%)

[2] OR = Odds Ratio

[3] AOR = Adjusted Odds Ratio

b)

Characteristic	EBOV positive, N = 125[1]	EBOV negative, N = 504[1]	Univariable regression			Multivariable regression		
			OR[2]	95% CI	Univariable p.value	AOR[3]	95% CI	Multivariable p.value
Study location					**<0.001**			**<0.001**
Kasese district (Bwera)	9 (4.6%)	185 (95.4%)	—	—		—	—	
Kasese district (Kagando)	33 (16.5%)	167 (83.5%)	4.06	1.96, 9.26		4.30	1.86, 10.7	
Arua district	58 (53.7%)	50 (46.3%)	23.8	11.6, 54.6		21.0	9.08, 53.5	
Gulu district	25 (19.7%)	102 (80.3%)	5.04	2.34, 11.8		5.83	2.60, 14.2	
Sex					0.11			**0.001**
Male	42 (24.0%)	133 (76.0%)	1.41	0.92, 2.14		2.37	1.41, 4.00	
Female	83 (18.3%)	371 (81.7%)	—	—		—	—	
Age					0.3			0.8
18 to 27	46 (17.8%)	213 (82.2%)	—	—		—	—	
28 to 37	33 (18.5%)	145 (81.5%)	1.05	0.64, 1.72		1.06	0.59, 1.89	
38 to 47	29 (27.1%)	78 (72.9%)	1.72	1.00, 2.92		1.49	0.79, 2.80	
48 to 57	11 (18.0%)	50 (82.0%)	1.02	0.47, 2.05		1.04	0.43, 2.41	
58 to 77	6 (25.0%)	18 (75.0%)	1.54	0.54, 3.91		1.39	0.43, 4.05	
Occupation					**<0.001**			0.4
Business	18 (11.5%)	138 (88.5%)	—	—		—	—	
Casual work	13 (14.0%)	80 (86.0%)	1.25	0.57, 2.66		0.96	0.39, 2.38	
Education	15 (18.8%)	65 (81.3%)	1.77	0.83, 3.73		1.74	0.75, 4.03	
Farming and animal handling	40 (18.8%)	173 (81.2%)	1.77	0.99, 3.29		1.14	0.59, 2.27	
Homemaker	39 (44.8%)	48 (55.2%)	6.23	3.30, 12.1		1.98	0.80, 4.90	

[1] n (%)

[2] OR = Odds Ratio

[3] AOR = Adjusted Odds Ratio

Table 2.3: **Univariable and multivariable logistic regression analysis of EBOV seropositivity against NP by occupational cadres** in a) HCWs and b) Community members.

tern of risk for study group and study location was noted, as above. Additionally, temporary housing significantly correlated with a higher odds of EBOV seropositivity (AOR = 2.36; 95% CI = 1.38-4.07; $p < 0.001$) compared to semi-permanent (reference) and permanent housing (AOR = 0.79; 95% CI = 0.51-1.25).

CCHFV seroprevalence

IgG seropositivity to CCHFV whole virus antigen (using the VectorBest assay) was detected in 256 out of 1353 participants (18.9%). CCHFV seropositivity was significantly higher in the Northern region (146/618; 23.6%), particularly in Arua district (105/306; 34.3%) (Figure 2.8a-c). HCWs had a significantly lower CCHFV seroprevalence (89/639; 13.9%) compared to community members (167/714; 23.4%; $p < 0.001$), driven by higher seropositivity in the community in the Northern region (Figure 2.8d-f). The seroprevalence for CCHFV exposure in different occupational cadres showed a similar pattern to EBOV; within the five most common occupations, the highest was detected in cleaners (20/102; 19.6%) within the HCW group and in homemakers (46/87; 52.9%) within the community group (Table 2.4).

Risk factors for CCHFV seropositivity

In the multivariable regression analysis (Figure 2.9b, Table A.5), CCHFV seropositivity was strongly associated with study location (Arua district: AOR = 4.67; 95% CI = 3.11-7.13; $p < 0.001$) and older age (highest AOR for the age group 58 to 77-year-olds (AOR = 2.95; 95% CI = 1.48-5.75; $p = 0.002$)). Being a HCW was strongly associated with lower odds of seropositivity (HCW: AOR = 0.42; 95% CI = 0.31-0.57; $p < 0.001$). Cleaners were the most exposed within the HCWs (AOR = 2.14; 95% CI = 1.07-4.22; $p = 0.057$), and homemakers within the community member analysis (AOR = 1.84; 95% CI

a)

Characteristic	CCHFV positive, N = 69[1]	CCHFV negative, N = 472[1]	Univariable regression			Multivariable regression		
			OR[2]	95% CI	Univariable p.value	AOR[3]	95% CI	Multivariable p.value
Study location					**0.011**			0.058
Kasese district (Bwera)	8 (7.0%)	106 (93.0%)	—	—		—	—	
Kasese district (Kagando)	28 (20.6%)	108 (79.4%)	3.44	1.56, 8.39		3.00	1.35, 7.40	
Arua district	17 (12.1%)	123 (87.9%)	1.83	0.78, 4.64		2.07	0.84, 5.51	
Gulu district	16 (10.6%)	135 (89.4%)	1.57	0.66, 4.00		2.02	0.79, 5.53	
Sex					0.8			0.14
Male	23 (13.4%)	149 (86.6%)	1.08	0.62, 1.84		1.65	0.85, 3.18	
Female	46 (12.5%)	323 (87.5%)	—	—		—	—	
Age					0.7			0.7
18 to 27	27 (14.6%)	158 (85.4%)	—	—		—	—	
28 to 37	15 (10.9%)	122 (89.1%)	0.72	0.36, 1.39		0.82	0.38, 1.72	
38 to 47	19 (14.3%)	114 (85.7%)	0.98	0.51, 1.83		1.07	0.51, 2.27	
48 to 57	7 (9.9%)	64 (90.1%)	0.64	0.25, 1.47		0.71	0.25, 1.82	
58 to 77	1 (6.7%)	14 (93.3%)	0.42	0.02, 2.21		0.31	0.02, 1.88	
Occupation					**0.046**			0.057
Nurse	30 (11.1%)	240 (88.9%)	—	—		—	—	
Cleaner	20 (19.6%)	82 (80.4%)	1.95	1.04, 3.60		2.14	1.07, 4.22	
Doctor	3 (5.8%)	49 (94.2%)	0.49	0.11, 1.45		0.43	0.10, 1.42	
Laboratory personnel	2 (6.3%)	30 (93.8%)	0.53	0.08, 1.89		0.50	0.07, 1.95	
Midwifery	14 (16.5%)	71 (83.5%)	1.58	0.77, 3.09		1.72	0.74, 3.93	

[1] n (%)

[2] OR = Odds Ratio

[3] AOR = Adjusted Odds Ratio

b)

Characteristic	CCHFV positive, N = 146[1]	CCHFV negative, N = 484[1]	Univariable regression			Multivariable regression		
			OR[2]	95% CI	Univariable p.value	AOR[3]	95% CI	Multivariable p.value
Study location					**<0.001**			**<0.001**
Kasese district (Bwera)	24 (12.4%)	170 (87.6%)	—	—		—	—	
Kasese district (Kagando)	36 (17.9%)	165 (82.1%)	1.55	0.89, 2.73		2.26	1.13, 4.56	
Arua district	63 (58.3%)	45 (41.7%)	9.92	5.66, 17.9		7.66	3.90, 15.5	
Gulu district	23 (18.1%)	104 (81.9%)	1.57	0.84, 2.92		1.56	0.80, 3.03	
Sex					0.6			0.9
Male	38 (21.7%)	137 (78.3%)	0.89	0.58, 1.35		1.04	0.63, 1.69	
Female	108 (23.7%)	347 (76.3%)	—	—		—	—	
Age					**<0.001**			**0.001**
18 to 27	47 (18.1%)	213 (81.9%)	—	—		—	—	
28 to 37	35 (19.7%)	143 (80.3%)	1.11	0.68, 1.80		1.03	0.59, 1.77	
38 to 47	38 (35.5%)	69 (64.5%)	2.50	1.50, 4.14		2.27	1.27, 4.07	
48 to 57	14 (23.0%)	47 (77.0%)	1.35	0.67, 2.60		1.48	0.68, 3.12	
58 to 77	12 (50.0%)	12 (50.0%)	4.53	1.90, 10.8		5.18	1.95, 13.8	
Occupation					**<0.001**			0.5
Business	26 (16.6%)	131 (83.4%)	—	—		—	—	
Casual work	17 (18.3%)	76 (81.7%)	1.13	0.57, 2.20		0.88	0.39, 1.98	
Education	9 (11.3%)	71 (88.8%)	0.64	0.27, 1.39		0.69	0.27, 1.62	
Farming and animal handling	48 (22.5%)	165 (77.5%)	1.47	0.87, 2.52		1.22	0.68, 2.20	
Homemaker	46 (52.9%)	41 (47.1%)	5.65	3.15, 10.4		1.84	0.82, 4.08	

[1] n (%)

[2] OR = Odds Ratio

[3] AOR = Adjusted Odds Ratio

Table 2.4: **Univariable and multivariable logistic regression analysis of CCHFV seropositivity for occupational cadres (measured with the VectorBest assay),** in a) HCWs and b) Community members.

= 0.82-4.08; $p = 0.500$) (Table 2.4). In an exploratory multivariable regression model (Table A.6), living in temporary housing significantly correlated with higher odds of CCHFV seropositivity (AOR = 2.97; 95% CI = 1.76-5.06; $p < 0.001$).

Seroprevalence of RVFV

RVFV IgG antibodies were detected in 19 out of 1,096 participants (1.7%). No significant difference in RVFV seroprevalence was noted in different regions or study groups (Figure 2.8). Neither within univariable nor multivariable regression models (Figure 2.9c, Table A.7). An exploratory multivariable regression model showed significance for a higher risk of exposure when rodents were present in the house (AOR = 3.73; 95% CI = 1.05-23.8; $p = 0.041$) (Table A.8).

2.6 Discussion

This study aimed to assess the risk of exposure to viral haemorrhagic fever viruses (VHFVs), among individuals across different occupational cadres, including roles within the general community and healthcare. Overall, we observed a very high seropositivity to both EBOV and CCHFV in the sampled Ugandan communities, with the highest risk occurring in the community rather than the healthcare setting and surprisingly was highest in an area with no recent reported VHFV cases in the last 50 years (Arua district).

The seroprevalence to EBOV as measured by ELISA directed against the EBOV NP was 16.3%. These results are towards the higher end of previous in-country estimates of seropositivity (0.9-16.6%) (Bower & Glynn, 2017; Nyakarahuka *et al.* 2020). This variation likely reflects heterogeneity in sampled areas, the use of different assays, and/or differences in exposure in risk

groups. We carried out our main analysis using an EBOV NP ELISA, due to high local vaccination rates with rVSV-ZEBOV, which contains EBOV GP as an antigen. The NP ELISA is known to be more cross-reactive than the GP ELISA (Natesan *et al.* 2016). However, GP and NP reactivity were not independent, suggesting that exposure induces responses to both antigens, albeit to differing magnitudes highlighted by the inter-individual variability. The weak correlation indicates antigen-specific immune heterogeneity, consistent with variable immunodominance or differential assay performance.

Geographical location was one of the strongest associations with EBOV exposure. There was significantly higher exposure in the Northern versus the Western region, the highest of which was in Arua district. This is notable as Arua district has never reported a case of Ebola virus disease (Figure 1.8), unlike all other study sites which were selected as sites where VHF had been previously reported. Arua is, however, situated only 34 kilometres away from a village (Ariwara) previously affected by EBOV within the DRC in 2019. The region has highly porous borders with shared cultural ties and frequent inter-border movement of residents and refugees. It is plausible that VHFV cases or outbreaks may have been unnoticed or misdiagnosed in this area (Ashraf *et al.* 2025). It is also plausible that cross-reactivity with another as yet unidentified filovirus may have driven these findings. Further studies are warranted, including incidence testing of acute febrile illness and sampling of bats and other wildlife sources in the area.

CCHFV seropositivity was also high in the sampled population (18.9%), in keeping with previous reports from Uganda (Atim *et al.* 2022). Exposure to CCHFV was highest in the Northern region, again in Arua district, even though there have been no CCHF cases reported from this area within the last 50 years (Figure 1.5). In December 2024, just after this study was conducted, a single CCHF case was reported in Arua City (personal communication, Stephen Balinandi). We have previously reported high seropositivity

in human and domestic animal populations for CCHFV in this region and have identified several nairoviruses in Rhipicephalus ticks, including CCHFV, Dugbe virus and Nairobi sheep disease virus (Atim, Ashraf, *et al.* 2023). There is a high potential for cross-reactivity following exposure to orthonairoviruses when testing for CCHFV by serology (Atim *et al.* 2022; Maze *et al.* 2025). Thus, human exposure to these viruses, earlier exposure and milder childhood infection, or misdiagnosis might explain the low number of symptomatic VHF cases in the area (Tezer *et al.* 2010). Antibodies against CCHFV are long-lasting (Hawman & Feldmann, 2023), and higher seroprevalence with older age suggests cumulative environmental exposure. There is likely significant under-reporting of CCHFV in this area.

We next explored the role of occupational exposure to VHFVs, within HCWs and communities. Importantly, we found that community members were at higher risk of exposure to EBOV and CCHFV than HCWs, suggesting that communities may not be adequately prioritised for vaccination and infection prevention and control (IPC) support, potentially allowing VHFV outbreaks to become more widespread.

We identified that homemakers (predominantly female) were most exposed within the community. An analysis of CCHFV cases in a centre in Afghanistan also identified housewives as a risk group of infection (Qaderi *et al.* 2021). In Uganda, it is common for homemakers to be the most involved in caring for unwell family members, preparing food for the family, and caring for peri-domestic livestock. Their exposures may be higher, for example, to bodily fluids of unwell individuals, vectors such as ticks, and the bodily fluids of animals potentially carrying VHFVs. Additionally, community members are likely to have received less formal training in vector and disease transmission and risk mitigation (Petrics *et al.* 2015). They also may not invest in preventative measures such as using acaricides that professional farmers use routinely. Therefore, sex, socioeconomic factors and education should be taken

into consideration when thinking about public health interventions.

Within the HCW group, cleaners were at the highest risk of exposure to both EBOV and CCHFV. While likely to be exposed frequently to both patients and bodily fluids, cleaners may be less likely to be well-trained in mitigation strategies and safe IPC. Given the risk of infection in hospitals, targeted training, adequate PPE and education are indicated to reduce the risk to cleaning staff.

Our finding that males have higher EBOV seropositivity differs from EBOV case analyses in other outbreaks, where the differences in infection rates between the sexes have been largely non-significant (Nkangu *et al.* 2017). This may reflect differences in exposure (i.e. different occupations) or risk behaviours and requires further investigation.

The majority of VHFVs, including EBOV and CCHFV, are zoonotic, and therefore, it is important to understand exposure in the context of animal reservoirs and vectors. Tick exposure is a known risk factor for CCHFV transmission (Hawman & Feldmann, 2023). In this study, however, the number of people reporting tick bites was unexpectedly low and may be affected by a lack of awareness about disease transmission risk.

While we found a high seroprevalence for EBOV and CCHFV in our study sites, we found a lower seropositivity for RVFV (1.7%) than in other studies (Nyakarahuka *et al.* 2018), highlighting differences in region and populations sampled or a difference in specificity/sensitivity between assays (Lapa *et al.* 2024). Rodents in the house correlated weakly with the risk of RVFV exposure on an exploratory analysis and may highlight the living situation of the participant being relevant to their exposure risk; this observation needs further investigation.

There were some limitations in this study. We used a cross-sectional design but did not randomise HCWs or community members to participate in the study.

Community members were chosen from villages 5km around health centres, but historical movements of both study groups were not recorded, which could lead to different environmental exposures. Finally, antibody waning may result in an underestimate of exposed, undiagnosed individuals. A small study looking at 15 survivors from the 2000 SUDV outbreak in Gulu showed that a third of the patients tested after 15 years had no IgG to SUDV nucleoprotein (Sobarzo *et al.* 2019).

2.7 Conclusions

In this study, we demonstrate clear evidence of high exposure rates to EBOV and CCHFV in Ugandan communities, particularly in the northwest of the country, which has not reported a single case of a VHFV in the last 50 years. We identified high-risk groups, including homemakers in the community setting and cleaners in the health care setting. Identifying geographical, social and occupational at-risk groups outside of VHFV outbreaks is essential to plan and trial preventative strategies before and during outbreaks. Hospital cleaners and homemakers in the communities may not have been sufficiently prioritised for intervention. Further studies should evaluate the effectiveness of PPE education and vaccination as an intervention in these groups.

Chapter 3

Identification of distinct environmental and socioecological areas in Uganda

3.1 Abstract

Previous research on Crimean-Congo haemorrhagic fever virus (CCHFV) highlights the importance of study location in determining exposure risks in Uganda. These risks may stem not only from environmental differences, such as rainfall, temperature, or land cover, but also from socioecological factors, which describe how humans interact with their surroundings. Identifying districts that are well-respresentative of the country as a whole, can be difficult without intense pre-surveying.

Using K-prototype clustering on 20 environmental and socioecological variables, 13 distinct Ugandan districts were identified to represent diverse risk profiles for CCHFV exposure. A qualitative study involving focus group discussions (FGDs) and key informant interviews (KIIs) was subsequently conducted in a subset of six districts.

The selected districts showed notable differences in climate, land use, proximity to wildlife, and their subregional locations within Uganda. Additionally, participants from both FGDs and KIIs described distinct living conditions and practices, further highlighting regional variation.

The selection of these diverse districts marks the first step in a broader research effort, including an extensive qualitative study on human-animal-tick interactions and a quantitative seroprevalence study on CCHFV exposure. This approach lays the foundation for deepening our understanding of CCHFV in Uganda, focusing on the country's varied ecological and social settings.

3.2 Acknowledgements

I conducted this site selection process with the initial support of Prof. Kimberly Fornace and Emilia Johnson, who introduced and continued to support me with spatial datasets and environmental variables.

The results presenting data from the FGDs and KIIs are part of the qualitative study from Chapter 4, and acknowledgements are highlighted in that chapter.

3.3 Introduction

Previous research (Atim, Niebel, *et al.* 2023; Lule *et al.* 2022), and the results from Chapter 2 highlight the importance of location regarding seroprevalence in humans and animals for CCHFV. There are major knowledge gaps surrounding how transmission and risk of exposure vary across districts. Identifying districts to study that are well-representative of the country as a whole can be difficult without intense pre-surveying.

Cluster analysis can be used as an approach to identify subgroups. First named

by Macqueen, 1967 in 1967, K-means cluster analysis is one of the most commonly used methods for clustering. It uses the partitional clustering approach, which partitions the dataset, rather than using a hierarchy (as in the hierarchical clustering approach) (Ikotun *et al.* 2023). The user supplies the number of clusters (k), and the algorithm randomly selects the cluster centres. The analysis is conducted multiple times, so that different centres are selected and optimal clusters identified. The algorithm generates the cluster's object mean value, and groups together ball-shaped clusters based on Euclidean distance (Ikotun *et al.* 2023).

To include categorical variables, an adapted variation of K-means clustering, the K-prototype algorithm, was developed by Huang, 1997 in 1997. It combines a K-mode algorithm developed for categorical datasets with the K-means algorithm. In the K-prototype algorithm, in addition to the Euclidean distance, a dissimilarity measure is added, facilitating the inclusion of variables with the option to calculate a mean, as well as categorical variables (Huang, 1997, 1998). The elbow method on the total within-cluster sum of squares (WSS) plot can be used to decide on the optimal number of clusters. WSS calculates the differences from each datapoint (in this case, each district) to each cluster centre. Where the decrease of WSS slows down, is the optimal number of clusters k.

Environmental and socioecological variables were added based on known factors influencing animal activities and possible tick abundances. Incorporating environmental and socioecological variables in the K-prototype analysis may enable greater capturing of sociodemographic factors and seroprevalence drivers to CCHFV in representative districts across the country. This method was used to design representative studies across Uganda, as described in the following Chapters 4 and 5. Sampling one random district within each cluster would be the ideal scenario to cover the environmental and socioecological diversity of Uganda.

This chapter aimed to identify distinct environmental and socioecological areas in Uganda, providing a solid foundation for the site selection process for further research on CCHFV and other diseases that may be linked to similar ranges of environmental and socioecological factors.

3.4 Methods

Various environmental (Table 3.1) and socioecological (Table 3.2) variables from different data sources were used within this chapter.

Environmental variables

Temperature and precipitation datasets were downloaded from WorldClim version 2.1 (Fick & Hijmans, 2017). This dataset presents climate data between 1970 and 2000, and is available with a resolution of approximately $1km^2$. Annual precipitation, isothermality, the mean temperature of the coldest quarter, and the mean temperature of the warmest quarter were incorporated into the analysis (Fick & Hijmans, 2017). The land coverage datasets, including crops, trees and urban coverage, were downloaded from Copernicus (Buchhorn *et al.* 2019). Copernicus Land Service uses satellite observations from 2015, and the data present a $100m^2$ resolution. Elevation data was downloaded directly within R, from the raster library (Hijmans, 2010) using the SRTM dataset (Jarvis *et al.* 2008) which presents elevation data in $1km^2$ resolution. This has recently been updated, but the geodata dataset is the same in the new library (Fick & Hijmans, 2017; Hijmans *et al.* 2024). Lastly, land surface temperature data from NASA satellites (June 2022) was downloaded from (NEO (Nasa Earth Observations), 2022). For all spatial datasets, the mean was calculated for each district to use in the K-prototype analysis. Examples are provided as maps in Figure 3.1, and all other graphs are presented in Appendix Figure A.1

and A.2.

Table 3.1: **Summary of environmental variables** used in the K-prototype analysis.

Variable	Description	Source
Annual precipitation	Spatially interpolated climate data from 1970 – 2000, with 1km^2 resolution	WorldClim (Fick & Hijmans, 2017)
Crops coverage	Spatial data at 100m^2 resolution from 2015	Copernicus Global Land Service (Buchhorn *et al.* 2019)
Elevation	Spatial data at 1km^2 resolution	Access through raster package (Hijmans, 2010; Jarvis *et al.* 2008)
Isothermality	Spatially interpolated climate data from 1970 – 2000, with 1km^2 resolution	WorldClim (Fick & Hijmans, 2017)
Land surface temperature	Spatial data from June 2022	NASA Earth Observations (NEO (Nasa Earth Observations), 2022)
Mean temperature coldest quarter	Spatially interpolated climate data from 1970 – 2000, with 1km^2 resolution	WorldClim (Fick & Hijmans, 2017)
Mean temperature warmest quarter	Spatially interpolated climate data from 1970 – 2000, with 1km^2 resolution	WorldClim (Fick & Hijmans, 2017)
Trees coverage	Spatial data at 100m^2 resolution from 2015	Copernicus Global Land Service (Buchhorn *et al.* 2019)

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Table 3.1 – *Continued from previous page*

Variable	**Description**	**Source**
Urban coverage	Spatial data at 100m^2 resolution from 2015	Copernicus Global Land Service (Buchhorn *et al.* 2019)

Socioecological variables

Data on border crossing districts, districts which contain national parks, and the fifteen sub-regions were collected from the Ugandan Ministry of Internal Affairs, publications, and Wikipedia entries (Uganda Ministry of Internal Affairs, 2022; Wasswa *et al.* 2020; Wikipedia, 2022a, 2022b). Districts identified as part of the cattle corridor, and districts with previous CCHFV outbreaks, were provided by Dr Stella Atim and Dr Steven Balinandi (personal communication). All animal density data (cattle, chicken, goat, pig, and sheep) were downloaded from the most recently available dataset FAO, 2015. An earlier version of the dataset from 2010 is described in Gilbert *et al.* 2018. The resolution of the animal density datasets is around 10km^2. Values from each district were combined, and the mean per district was used for the K-prototype analysis. Finally, population density was downloaded from WorldPop and CIESIN, 2018. This dataset from 2020 provides population density estimates using Random Forest-based methods, with a resolution of around 1km^2. Log-transformation was conducted to normalise the distribution, and mean values per district were used in subsequent analysis. Examples are demonstrated as maps in Figure 3.2, and all other graphs are presented in Appendix Figure A.3 and A.4.

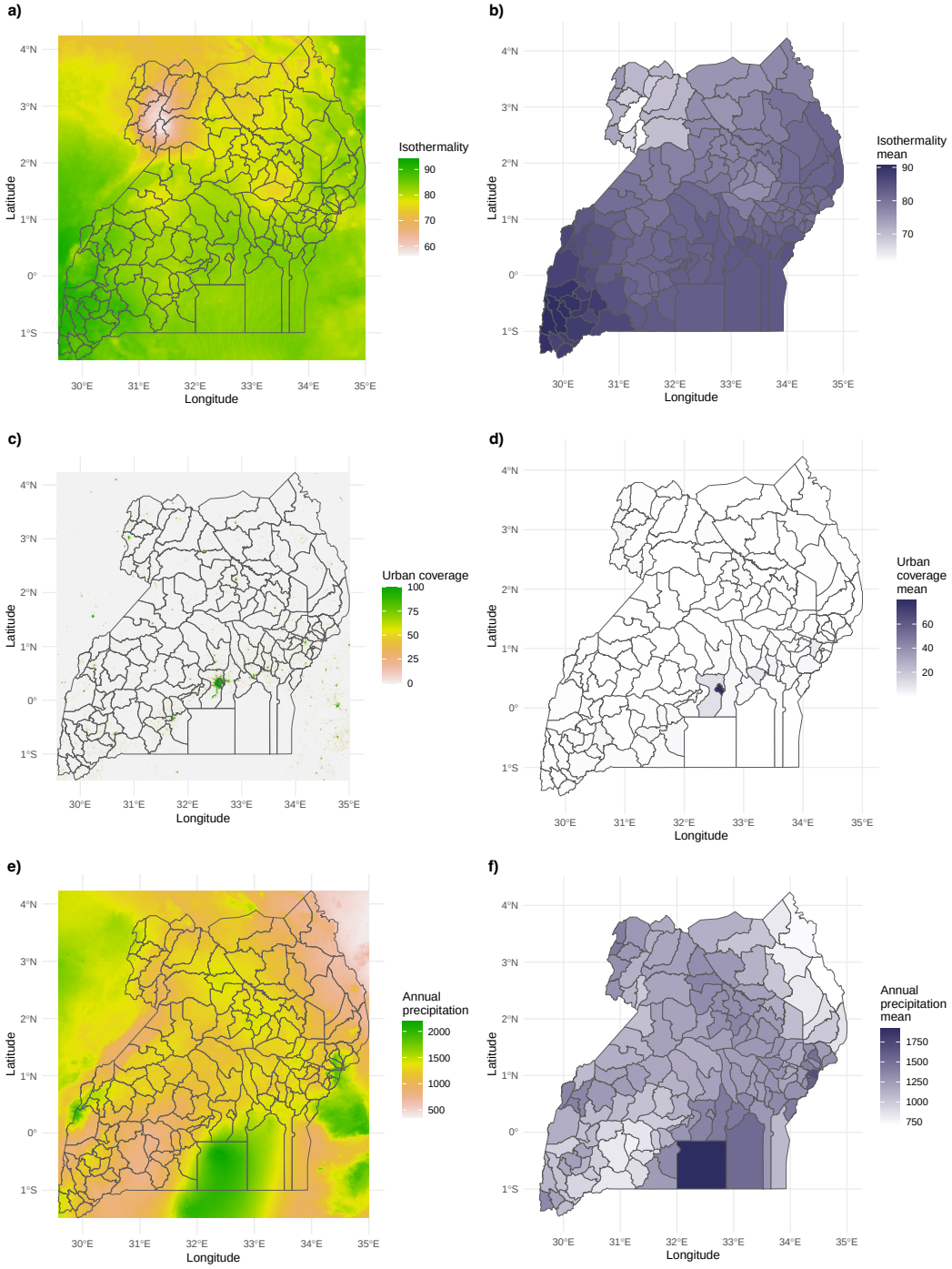

Figure 3.1: **Example subset of environmental variables** used for the K-prototype analysis. All maps represent Uganda and the 136 districts. **(a)** Isothermality from Fick and Hijmans, 2017, **(c)** urban coverage from Buchhorn *et al.* 2019, and **(e)** annual precipitation from Fick and Hijmans, 2017. In **(b)**, **(d)** and **(f)**, the respective mean-per-district for the variables, as used in the K-prototype analysis.

Table 3.2: **Summary of socioecological variables** used in the K-prototype analysis.

Variable	Description	Source
Border crossing	Districts with border crossings to all neighbouring countries	In country data (Uganda Ministry of Internal Affairs, 2022; Wasswa *et al.* 2020)
Cattle corridor	Historical data where cattle rearing and mixed farming practices are common	Dr Stella Atim and Dr Steven Balinandi (personal communication)
Cattle density	Modelled data from 2015; $10km^2$ spatial resolution	FAO, Gridded Livestock of the World (FAO, 2015; Gilbert *et al.* 2018)
Chicken density	Modelled data from 2015; $10km^2$ spatial resolution	FAO, Gridded Livestock of the World (FAO, 2015; Gilbert *et al.* 2018)
Goat density	Modelled data from 2015; $10km^2$ spatial resolution	FAO, Gridded Livestock of the World (FAO, 2015; Gilbert *et al.* 2018)
National Park	Districts with National Parks within their borders	In-country data (Wikipedia, 2022a)
Pig density	Modelled data from 2015; $10km^2$ spatial resolution	FAO, Gridded Livestock of the World (FAO, 2015; Gilbert *et al.* 2018)
Population density	Spatial estimated population data from 2020; $1km^2$ spatial resolution	WorldPop (WorldPop & CIESIN, 2018)

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Table 3.2 – *Continued from previous page*

Variable	**Description**	**Source**
Previous outbreaks of CCHFV	Recorded CCHFV outbreak districts between 2000 and 2022	Dr Stella Atim and Dr Steven Balinandi (personal communication)
Sheep density	Modelled data from 2015; 10km^2 spatial resolution	FAO, Gridded Livestock of the World (FAO, 2015; Gilbert *et al.* 2018)
Sub-region	15 sub-regions separating the four big regions of Uganda	In-country data (Wikipedia, 2022b)

All work was conducted using R 4.2.0 (R Core Team, 2021). The raster library was discontinued during the study, and all spatial data was handled with the terra library (Hijmans, 2020). Ggplot2 (Hadley Wickham, 2016) was used for the creation of maps and graphs.

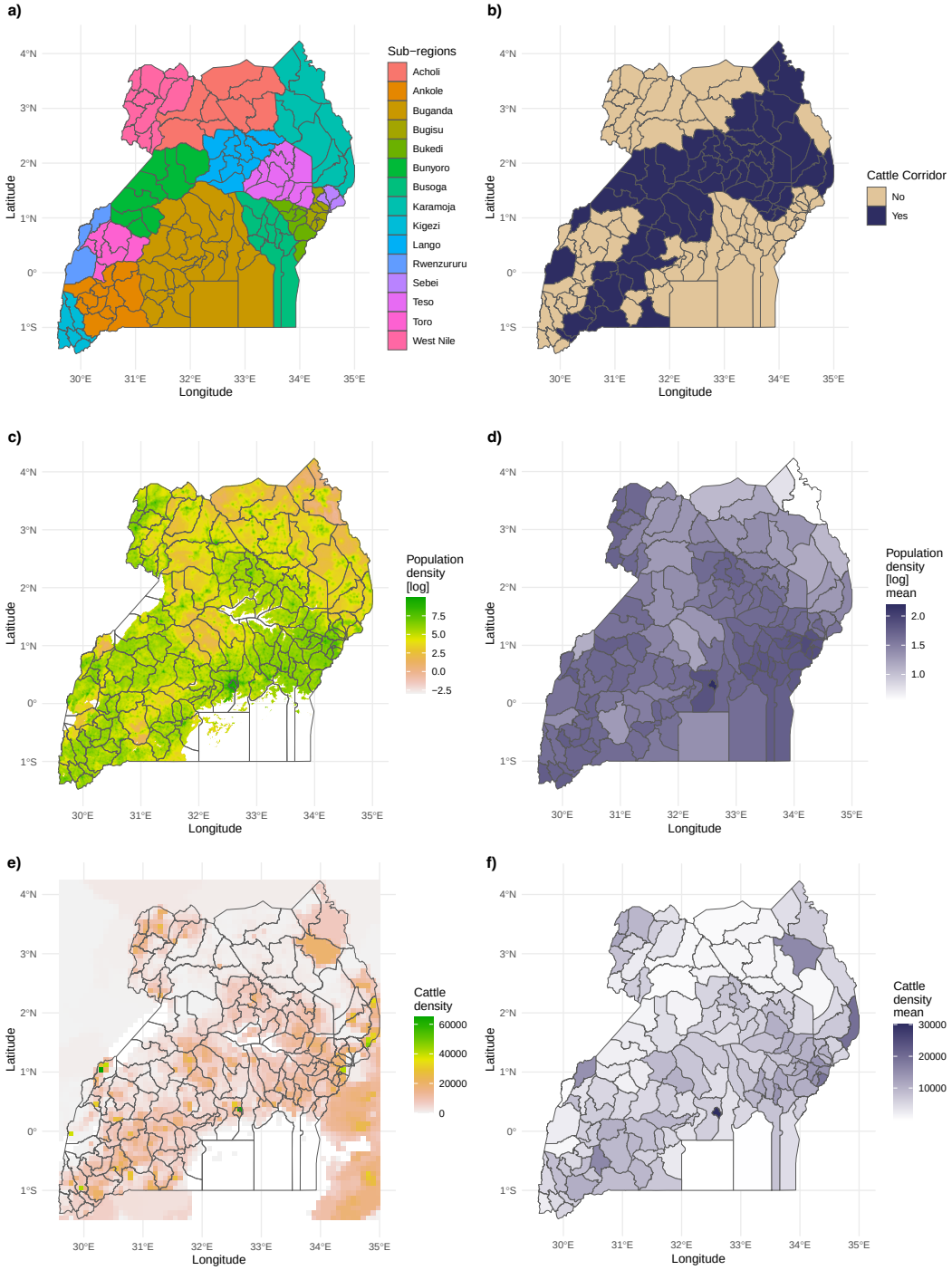

Figure 3.2: **Example subset of socioecological variables** used for the K-prototype analysis. All maps represent Uganda and the 136 districts. **(a)** 15 sub-regions of Uganda. **(b)** Districts within the cattle corridor. **(c)** Population density (WorldPop & CIESIN, 2018) in log-transformation as spatial representation and in **(d)** as mean-per-district as used in the K-prototype analysis. **(e)** Cattle density from FAO, 2015 and plotted in **(f)** as mean-per-district.

Identification of subgroups using cluster analysis

Clustering analysis was used to identify distinct district subgroups to design representative surveys nationwide. As the dataset contains a mix of categorical and numerical variables, a K-prototype algorithm was used (Huang, 1997, 1998).

The clusterMixType library from Szepannek, 2019 was employed to conduct the K-prototype analysis. First, all categorical variables were checked to be factoral, and all numerical variables were scaled. Scaling was carried out, as the numerical variables are all spatial datasets with a large span of values. A function scale was deployed from the terra library (Hijmans, 2020), which subtracts the mean of the variable from each datapoint. This brings all variables closer together and prohibits uneven contribution of variables to clustering. The elbow method was used to decide the optimal number of clusters (k). Numbers between 1 and 30 are used to calculate the WSS, which is repeated 25 times to calculate a mean and standard deviation. The optimal number of clusters is decided where the decrease of WSS slows down. The R script is presented in Appendix A (p. 191).

A table to present the differences within the selected districts was created with (Cheng *et al.* 2024; Ren & Russell, 2021).

Methodology for qualitative and quantitative studies

A qualitative study was carried out initially to assess likely associations with risk of exposure to CCHFV. FGDs and KIIs were conducted in communities within each of the finally selected six districts, with diverse members of the communities, including multiple occupations, education levels, a range of ages and both sexes. A detailed synopsis of the methods used is described further in Chapter 4. In this chapter, the data generated on district variability is

presented, which highlights an analysis and quotes from participants who were asked to describe their home surroundings. Additionally, pictures show each of the districts in snapshots.

Continuing from the qualitative assessment, a quantitative study was developed and carried out in four districts. Detailed methodology and results are presented in Chapter 5. Here, the results focused on descriptions of the districts are represented.

3.5 Results

Data on nine environmental and 11 socioecological factors were successfully obtained and incorporated into the K-prototype analysis. An ideal number of 13 clusters was estimated by plotting the WSS (Figure 3.3). This number separates distinct areas of Uganda by both environmental and socioecological factors (Figure 3.4), and maximises the variation in zones surveyed.

However, to incorporate the feasibility of recruitment and time constraints during my PhD, a subset of 6 districts was selected for a detailed risk analysis. This included Kampala, Kalangala, Kasese, Arua, Soroti and Kaabong (Figure 3.5). These districts were selected from the original 13 for pragmatic reasons (availability of study teams and acceptable security assessments). Throughout this chapter and later on, I present them in the following order: Kampala, Kalangala, Kasese, Arua, Soroti and Kaabong.

The differences within the selected districts are presented in Table 3.3. Multiple sub-regions and all regions are covered in the selection. Some districts contain border crossings or national parks, while others do not. As described in the introduction, the 'cattle corridor' was a historical tool with still a considerable influence, and our selection shows some districts within and some outside it. Kampala has the highest population density and urban coverage,

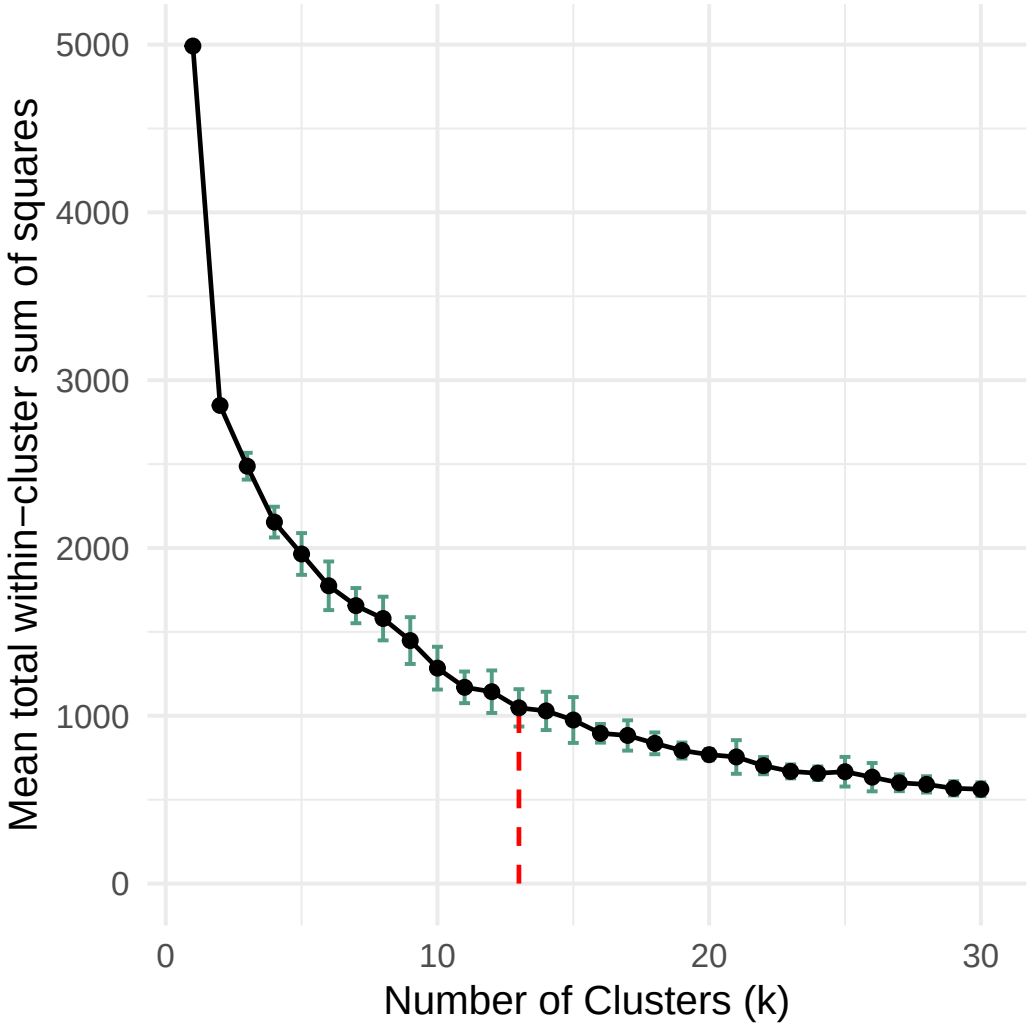

Figure 3.3: **Mean total within sum of squares (WSS),** after K-prototype analysis with various numbers of clusters (k). The analysis was run 25 times to calculate the means with standard deviations. The red dashed line shows the optimal number of clusters (13), using the Elbow method.

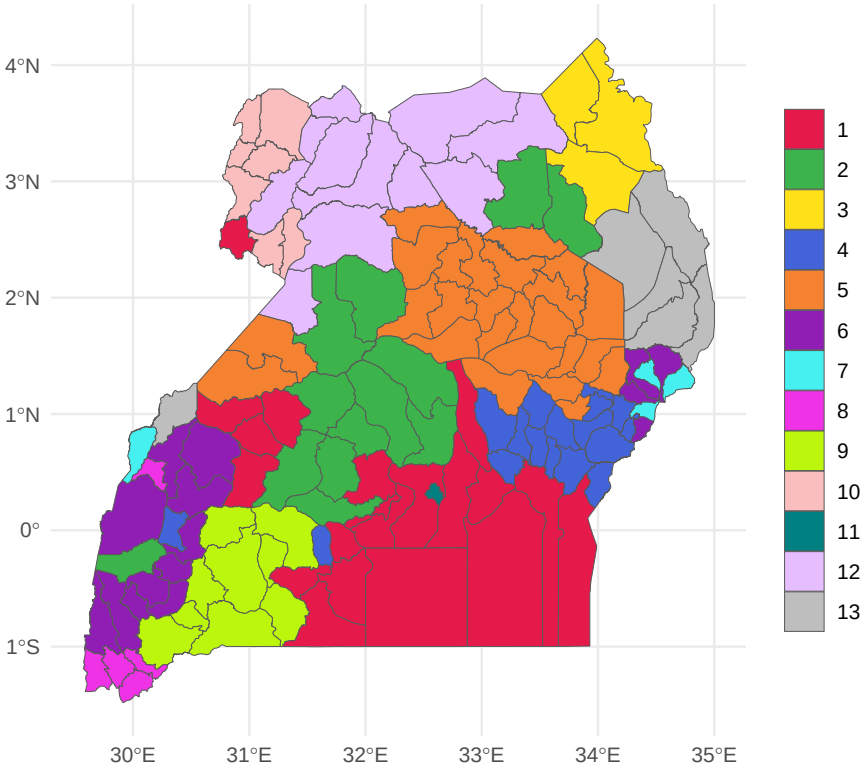

Figure 3.4: **13 clusters.** Created with K-prototype analysis, including 20 environmental and sociological variables.

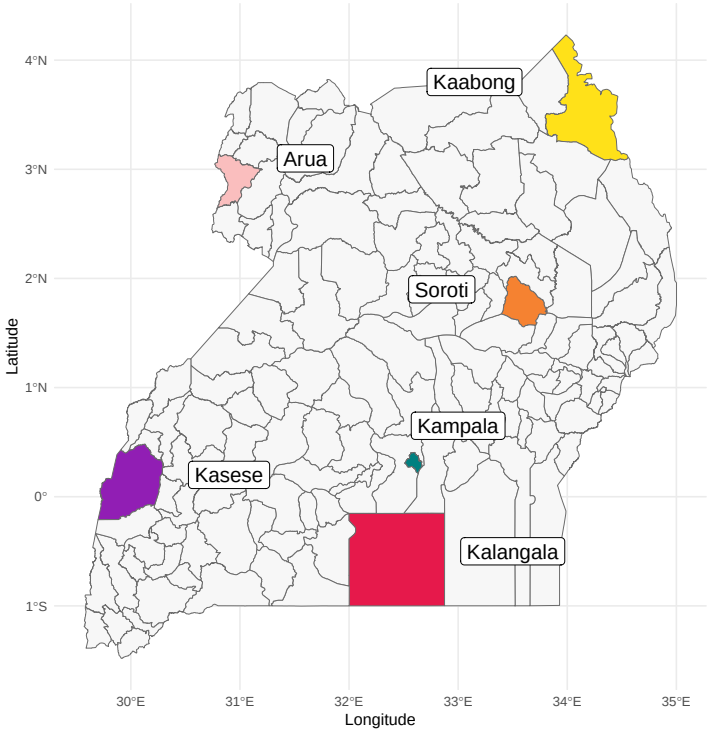

Figure 3.5: **6 selected study districts for the risk analysis substudy.** Selected through initial K-prototype analysis to present distinct areas within Uganda.

	Part of 'Cattle Corridor'	Previous CCHF outbreak cases	Region	Sub-Region	Border crossing	National Park	Population density	Elevation
Kalangala	no	no	central	Buganda	no	no	3.93	1136.11
Kaabong	yes	no	northern	Karamoja	yes	yes	1.76	1476.50
Soroti	yes	no	eastern	Teso	no	no	5.38	1074.39
Kasese	yes	no	western	Rwenzururu	yes	yes	4.94	1480.34
Arua	no	no	northern	West Nile	yes	no	5.49	1132.61
Kampala	no	yes	central	Buganda	no	no	9.02	1180.90

	Urban coverage	Crops coverage	Cows abundance	Pigs abundance	Sheep abundance	Goat abundance	Chicken abundance
Kalangala	0.03	0.95	37.66	6.82	68.96	307.42	506.27
Kaabong	0.10	5.75	5233.11	411.01	2431.11	6907.93	12004.98
Soroti	1.04	34.85	7902.39	1784.55	894.47	6201.59	18993.76
Kasese	0.98	23.12	3196.97	420.29	547.77	9345.19	38494.10
Arua	3.50	29.89	6106.99	1590.59	867.80	13164.47	32733.55
Kampala	79.77	2.65	28468.68	5441.38	12889.50	40590.98	738168.21

	Tree coverage	Land surface temperature	Annual precipitation	Temperature of warmest quarter	Temperature of coldest quarter	Isothermality
Kalangala	3.04	183.28	1920.02	22.30	21.09	82.82
Kaabong	18.17	185.00	750.60	23.08	20.31	77.81
Soroti	15.87	194.70	1286.65	25.82	23.14	76.85
Kasese	27.18	176.58	1151.93	20.47	19.61	86.96
Arua	15.71	192.88	1318.78	25.15	22.11	71.16
Kampala	2.94	189.86	1325.93	22.45	20.76	81.83

Table 3.3: **Characteristics of selected Districts.** All variables entered into the K-prototype analysis and their levels in the six selected districts. Blue gradient shows the highest value in dark blue, with white being the lowest level. Each region has been added for context, but was not added to the final analysis as a unique variable, as the subregion contains similar information.

as the capital city of Uganda. The estimated animal abundance is the highest in Kampala. Environmental variables vary, with different districts at the highest or lowest positions.

The six study districts vary greatly in their socioecological and environmental variables (Table 3.3), as well as in the descriptions provided by the study participants during the qualitative study. Further below, each selected district is described with population numbers, land size, estimated per capita gross domestic production (GDP), climate and animal density. Additionally, reports from participants in the FGDs and KIIs were used to describe the districts. This helps to contextualise the studies in Chapters 4 and 5, which were conducted in the following districts.

Kampala District

Kampala is the capital of Uganda and the largest city with around 1.8 million inhabitants and more entering during the daytime (Uganda Bureau of Statistics, 2024) (see Table 3.4 for comparison between the districts). It has the highest population density in Uganda and is predominantly urban (Figure 3.6a), with a land size of 73 sq mi. It is part of the central region, lying on the shores of Lake Victoria. Uganda's international airport is located in Entebbe, a neighbouring town connected by a highway. It comprises five divisions, of which Kawempe Division was selected for the study.

Pardee *et al.* 2017 estimated the GDP per capita for each district, where Kampala has the second largest GDP per capita ($ 2,655). GDP describes the total goods and services produced annually, and is used to compare economic progress (Pardee *et al.* 2017). While the GDP is relatively high, the participants in the FGDs describe the reality for most people in Kawempe as congested living situations with dirty water trenches, that often contained rubbish, faeces and used condoms. A participant in the Community leader

District	Population	Land area (total area) [sq mi]	GDP per capita	Classified climate
Kalangala	74,000	180.8 (3,514.7)	$ 67	Tropical monsoon
Kaabong	265,000	2,789.1	$ 75	Tropical savanna
Soroti	266,000	545.1	$ 586	Tropical savanna
Kasese	854,000	458 (1,052)	$ 540	Tropical savanna
Arua	160,000	1,249.6	$ 261	Tropical savanna
Kampala	1,800,000	73	$ 2,655	Tropical rainforest

Table 3.4: **District population, land area, GDP per capita and climate.** Population numbers are taken from Uganda Bureau of Statistics, 2024. Land area is recorded from Wikipedia entries (Wikipedia, 2022a), and shows additional total areas for Kalangala (the land area is much smaller than the total area, as they are islands in a large area of Lake Victoria), and Kasese (contains two large National parks which are not used by its human population). The estimated GDP per capita is recorded from Pardee *et al.* 2017, and the climate classification is the classification by Koeppen (Beck *et al.* 2018; Koeppen, 1884).

FGD said that *"we all live in a slum"*.

Kampala's climate is classified as tropical rainforest (Beck *et al.* 2018; Koeppen, 1884) with consistently high temperatures between 17 and 26°C, and abundant rainfall during the year. It does not contain a national park, nor a large wilderness, except for a few swamps towards Lake Victoria.

Domestic animals are rare, as a participant explains *"it's hard for someone to rear an animal in a rental"*. We saw a few goats walking on the side of the road (Figure 3.6b), and a few people reported owning dogs, cats and pigs.

> *"Here in Kampala, we don't have [animals] but some of us have them in villages." (Men FGD Kampala)*

Even if a few people keep animals with them in Kampala, some report that they own animals in their home villages, in rural Uganda. Additionally, a participant from the KIIs mentioned the massive consumption of livestock and slaughterhouses in Kampala, to feed especially the growing middle class, who *"feed on protein and more protein. And they need it, yet they don't graze it from here"*.

Kalangala District

Kalangala district comprises 84 islands in Lake Victoria (Figure 3.7a), and is part of the Central region, within the Buganda subregion. The island on which all FGDs and KIIs were conducted is the largest and main island, Bugala Island. In 2024, around 74,000 people lived on the islands (Uganda Bureau of Statistics, 2024), on a total land area of 180.8 sq mi. Participants describe strong differences between the islands, some being more congested than others.

The GDP per capita in Kalangala is $ 67 (Pardee *et al.* 2017). Fishing was described as the primary source of income and is still widely practised,

a)

b)

Figure 3.6: **Kampala.** Both pictures were taken in Kyebando, Kampala, in October 2023.

even with challenges such as the scarcity of fish and harsher conditions. Most people stay in so-called "landing sites", which are fishing villages directly on the shores. They are described by participants of FGDs and KIIs as *"slum-like areas"*, characterised by congestion, a lack of toilets, and temporary housing.

Kalangala's climate is classified as tropical monsoon (Beck *et al.* 2018; Koeppen, 1884), which has a distinct wet season with heavy rainfalls. There are no national parks on the islands, but some protected forests. Participants mentioned forests which used to cover all islands, but have been extensively cut down to make space for palm oil plantations (Figure 3.7b) and to use the wood for charcoal production.

Many people reported having a few animals around their homestays. But as mentioned, fishing is common and fish are the main source of protein in most communities.

Kasese District

Kasese district is part of the Western region of Uganda and the Rwenzururu sub-region. It has a land size of 458 sq mi and 854,000 inhabitants. It borders the Democratic Republic of the Congo (DRC) to the west, and has regular border traffic in and out of the DRC.

The GDP per capita is $ 540 (Pardee *et al.* 2017), with agriculture being the main activity. Participants describe their home district as friendly and as a *"fair living environment"* where they *"have houses, cars, fields, [and football] pitches."*

The climate in Kasese is classified as tropical savanna (also known as tropical wet and dry) (Beck *et al.* 2018; Koeppen, 1884). It is characterised by seasonality with dry and wet seasons. There are two large national parks within its borders, the Queen Elizabeth National Park and the Rwenzori Mountains

a)

b)

Figure 3.7: **Kalangala District.** a) View over Kalangala town on Bugala Island. b) Palm oil plantations on the roadside, driving on Bugala Island. Both pictures were taken in October 2023.

National Park (Figure 3.8a). Participants mentioned a wide range of wild animals, including chimpanzees, baboons, lions, elephants, wild dogs, and more. In addition to the wild animals, the River Nyamwamba (Figure 3.8b) coming down from the Rwenzori Mountains is often discussed and mentioned in a KII to *"sometimes disturb the people's living environment"*, due to strong floods.

As described above, the main industry is agriculture, and also the women in their FGD mention many domestic animals around their homesteads.

Arua District

Arua district lies in the Northern region as part of the West Nile sub-region. It has a land size of 1,249.6 sq mi and 160,000 inhabitants. It borders the DRC to the West and is close to South Sudan in the North, and is a first entry point for many refugees due to its location. A participant in a KII described Arua as a *"business hub where many trucks come from different places and the population is high"*. In 2020, Arua City was elevated to city status and is now separate from Arua District in governmental administrative terms. However, for this work, they were assessed together.

The GDP per capita is $ 261. Participants described having mixed housing, including some permanent houses, some semi-permanent structures, and some grass-thatched temporary houses (Figure 3.9a).

Arua is classified to have a tropical savanna climate (Beck *et al.* 2018; Koeppen, 1884), and participants described the area to be often dry with more extended periods of drought. When the rains start, the participants mentioned they can be heavy with occasional flooding in some parts. A few kilometres from town is a large game reserve called Ajai.

Most participants owned animals and kept them around their homes, including cattle, goats, and chickens (Figure 3.9b).

a)

b)

Figure 3.8: **Kasese District.** a) View over Kasese District with the plains of Queen Elizabeth National Park and Lake George in the middle, and the start of the Rwenzori Mountains in the back. b) River Nyamwamba. Both pictures were taken in October 2023.

a)

b)

Figure 3.9: **Arua District.** a) Village setting outside of town, featuring grass-thatched houses. b) Trees and animals around homesteads. Both pictures were taken in November 2023.

Soroti District

Soroti district is part of the Eastern region and the Teso sub-region. It has a population of 266,000 people and a land area of 545.1 sq mi. Like Arua city, Soroti city was elevated to city status in 2021, but was assessed together within this work.

The estimated GDP per capita for Soroti is $ 586 (Pardee *et al.* 2017). Soroti city is an urban centre with a good infrastructure (Figure 3.10a), but some areas were considered to be slums by the participants.

Soroti's climate is classified as tropical savanna. There are no national parks in the district, but Lake Kyoga is partly in Soroti with large swampy areas around its shores (Figure 3.10b). Trees were and are cut down heavily for making charcoal, as a participant in the KII explained: *"Apparently, charcoal here also supplies Kampala"*.

A participant from the KIIs explained that the people of this area *"are mainly cattle keepers, but of course, due to trends and due to increased population, issues of land have become a very big issue"* in recent years. Most participants described raising a few animals at their homes.

Kaabong District

Kaabong district lies within the Northern region of Uganda and the Karamoja sub-region. It borders South Sudan to the North and Kenya to the East. The land area is 2,789.1 sq mi and is sparsely inhabited by only 265,000 people (Figure 3.11a).

The estimated GDP per capita is $ 75 (Pardee *et al.* 2017), and Kaabong district, together with other districts in the Karamoja sub-region, has one of the highest poverty rates in the country, with low literacy, access to grid

a)

b)

Figure 3.10: **Soroti District.** a) View over Soroti town. b) Swamps within Soroti District. Both pictures were taken in November 2023.

electricity or mobile phone ownership (Uganda Bureau of Statistics, 2024). Most houses are reportedly built with grass and mud (Figure 3.11b).

Kaabongs's climate is classified as a tropical savanna (Beck *et al.* 2018; Koeppen, 1884). Most participants from the FGDs and KIIs reported the presence of dry, scanty vegetation with little rainfall and prolonged dry seasons. The district contains the large Kidepo Valley National Park and Timu Central Forest Reserve. Wild animals regularly move close to villages, even as far as Kaabong town.

> *"If you are to move right now for the next about one or two kilometres, you will meet Elephants loitering around the rivers." (KII Kaabong)*

Animal rearing is the primary activity in Kaabong. Many reasons for this are mentioned, including the traditional way of living, in part due to the dry climate. People often owned large herds of animals. Additionally, the *"bad practice of raiding"* (KII Kaabong) is a practice commonly seen. This is when men go from one village to another village in the middle of the night to steal cattle and goats. The study team was informed about a neighbouring village that had been raided just the day before the team left the district.

a)

b)

Figure 3.11: **Kaabong District.** a) View towards Kaabong town. b) Grass thatched house from Kaabong district. Both pictures were taken in March 2024.

3.6 Discussion

Here, I used K-prototype analysis to identify clusters based on diverse environmental and socioecological factors to enable the selection of a subset of districts to survey that would represent a diverse range of these factors. Thirteen distinct clusters were identified, of which a subset of 6 distinct districts in Uganda were selected for further studies on CCHFV. The analysis assured that a variety of distinct zones (based on environmental and socioecological variables) in Uganda were represented. K-prototype analysis was conducted, including 20 environmental and socioecological variables, to identify 13 distinct district clusters within Uganda, of which a subset of 6 districts (from 6 clusters) was chosen. A good range of regions, climates, and population and domestic animals densities is represented, also confirmed by the descriptions of the study participants in the districts.

The limitations of the K-prototype analysis relate predominantly to the input datasets. Studies modelling tick abundance, such as Lule *et al.* 2022 and Okely *et al.* 2020, employed larger datasets, tested for correlations and tried out different models before deciding on the final model. Additionally, some variables are somewhat outdated, including tree and urban coverage and domestic animal densities modelled by Gilbert *et al.* 2018, which would benefit from an updated dataset. Another issue with the domestic animal densities is that Kampala has the highest values in all domestic animal densities, although few animals are reared within the city borders. This is due to the modelling process, which includes population density to estimate the animals needed to feed the population. They have a cut-off to correct for this in large cities; however, Kampala was just below the cut-off, and therefore still shows the high densities, which overestimate the animal density and thus possible exposure risk to zoonotic diseases. The WorlClim dataset only covers a large timeframe, which might not represent climate change or population changes occurring more recently in Uganda. This could be improved with more nuanced datasets within

the year of analysis. However, the overall aim of this chapter, to identify distinct areas in Uganda, was not likely to have been strongly affected by these aspects, as it is a large-scale clustering, not driven by small changes, but rather looking for broader, more general patterns.

Some areas within Uganda were missed from this first-stage analysis, due to the selection of six districts. More districts will be added in future work. The total of 13 sites will be studied to evaluate ecological drivers of CCHFV infection over the next 3 years as part of a larger EEID-funded study.

The district selection process was carried out to enable the following qualitative and quantitative studies in Chapter 4 and 5 to be performed in study sites that differ significantly in various environmental and socioecological variables. They represent larger areas within Uganda to conduct representative surveys on CCHFV exposure risk. K-prototype analysis is an effective method for integrating many complex variables. It uses a minimally biased system to cluster similar districts, allowing for selecting distinct ones from each cluster.

Chapter 4

Decoding Crimean-Congo haemorrhagic fever virus transmission: exploring human-animal-tick interactions across six districts in Uganda

4.1 Abstract

Crimean-Congo haemorrhagic fever virus (CCHFV) causes a viral zoonotic disease transmitted through tick bites and direct contact with infected blood or tissue. Socioecological and behavioural risk factors for CCHFV exposure in Uganda are poorly understood.

To explore human-animal-tick interactions across Uganda, we conducted 24 focus group discussions (FGDs) and 31 key informant interviews (KIIs), in six environmentally and socioecologically diverse districts, between late 2023 and early 2024. FGDs were conducted in groups of community leaders, men,

women and teenagers. Medical doctors, veterinarians, traditional healers, district surveillance officers, and herdsmen were interviewed as key informants. Data were next translated, transcribed, and analysed using iterative categorisation.

Most people that we interviewed experienced tick bites, some as frequently as every day. Close contact with animals was common, including cohabitation, largely due to concerns about animal theft. Less frequent but notable practices included slaughtering animals for consumption or sacrifice, and interactions with wild animals during hunting. Slaughtering and butchering were reported if an animal was sick or had died. Plucking and roasting engorged ticks was a practice described in the Kaabong and Arua districts of Northern Uganda.

These practices and behaviours highlight key risks of transmission of CCHFV and underscore the need for future studies to address these specific behaviours, quantifying the extent of the associated risk, in order to identify targeted and culturally appropriate interventions. These should be planned while considering different underlying reasons for the behaviours, while addressing the risk for zoonotic diseases like Crimean-Congo haemorrhagic fever (CCHF).

4.2 Acknowledgements

This study involved many people. A complete acknowledgement is presented in Table 4.1.

This study was solely funded by my personal grant as part of the Wellcome Trust PhD funding (218518/Z/19/Z).

	Contributions
Marina Kugler	Setting up of the study, including ethics, study planning, protocol, and topic guides Leading recruitment in all districts Conducting a few KIIs Developing codebook, coding, conducting iterative categorisation, analysing and writing
Lazaaro Mujumbusi, Lucy Pickering	Helping during setup, guiding through the topic guide and codebook development, and mentoring during analysis
Janet Seeley, Stella A Atim, Chris Davis, Emma C Thomson, Poppy H L Lamberton	Helping during setup and oversight of the study
Lazaaro Mujumbusi, Richard Muhumuza, Titus Apangu, Evalyne Umo, Mathias Akugizibwe, Edward Obicho	Conducting FGDs and KIIs Translating and transcribing all recordings
Richard Muhumuza, Mathias Akugizibwe	Coding

Table 4.1: Contributions to AVQ study.

4.3 Introduction

Crimean-Congo haemorrhagic fever virus (CCHFV) is a zoonotic virus, that can infect domestic and wild animals and is thought to be spread mainly by tick bites as well as directly through contact with infected animals or other human cases (Hawman & Feldmann, 2023; Hoogstraal, 1979). Therefore, understanding interactions of humans with ticks and animals is essential for delineating risk factors for exposure to CCHFV.

Previous risk analyses evaluating exposure to CCHFV provided initial variables to explore, based on previous research. These include common behaviours, such as caring for animals, slaughtering animals, and hunting. However, specific behaviours and activities regarding these activities and interactions with animals, ticks and animal products vary throughout the world, and may even be different in neighbouring communities.

Multiple studies have investigated risk factors for CCHFV infections and exposure in Uganda (Atim *et al.* 2022; Balinandi *et al.* 2022; Mirembe *et al.* 2021). However, only Atim *et al.* 2022 included the practice of eating engorged ticks in their survey, which emerged as a significant risk factor for exposure to CCHFV. Eating engorged ticks is not limited to Uganda. A recent study in Cameroon reported 3% of interviewees had eaten ticks (Gasparine *et al.* 2025). This previously underappreciated behavioural risk factor may in part explain the presence of higher antibody titers in Arua than in Gulu and Kasese (Chapter 2). Further factors are also likely to be under-reported and may be key to better understanding exposure risk and opportunities for intervention.

Few previous qualitative studies have been conducted regarding CCHFV and other tick-borne diseases. The first-ever qualitative study on CCHFV in Uganda was published in 2023 by Ayebare *et al.* 2023, and focused on knowledge, attitudes, and control measures. However, it only encompassed one focus group discussion (FGD). Nevertheless, it provided evidence that interviewed

participants felt at risk of getting infected with CCHFV due to *"close contact with ticks and animals"* (Ayebare *et al.* 2023). In this study, participants mentioned that they slaughtered animals at home and that some ate half-cooked meat. This study, while useful, was conducted in Kagadi district of Uganda (Western Region), and did not include comparisons with other areas in Uganda. Therefore, the extent of such behaviours and how people interact with animals and their meat was previously not well understood on a wider scale.

In another study called the COHRIE project, which involved collaboration between researchers from the Uganda Virus Research Institute (UVRI) and LSHTM, risk factors associated with CCHFV and brucellosis at the human-livestock-wildlife interface in Uganda were recently investigated, and the protocol is described in a preprint publication (Kizito *et al.* 2024). This project included a multidisciplinary approach, including qualitative evaluation of risk (Agaba *et al.* 2025). The focus of this study was to highlight sex roles and different exposure pathways to zoonoses. Clear sex roles were described, including men and boys caring for larger livestock outside the home, and women and girls dealing with household-related work. They call for sex directed interventions and highlight the need for contextualization.

Further work around knowledge, attitude and practices (KAP) on CCHFV has been carried out by targeted surveys rather than through qualitative interviews or discussions. Ahmed *et al.* 2021 surveyed within healthcare professionals in Pakistan and Ilboudo, Dione, *et al.* 2025 within mixed crop-livestock farmers in Burkina Faso, where several risk factors were identified. Male sex and coming from households owning livestock grazing areas were linked with an especially high risk.

Egwuenu *et al.* 2025 published recently on CCHFV in Nigeria, conducting a One Health joint risk assessment, to identify infection routes within the country with local experts. A similar approach was conducted by Namgyal *et*

al. 2022, who carried out a qualitative risk assessment of the introduction of CCHFV in Bhutan.

Other qualitative studies on ticks and tick-borne diseases have been carried out in the Northern Hemisphere (Bowser *et al.* 2025; Slunge & Boman, 2018). Cameron *et al.* 2021 investigated perceptions of Lyme disease risk in communities with high and low levels of self-reported concern regarding climate change. The study concluded that public health messaging about Lyme disease should be decoupled from climate observations in climate-sceptic audiences.

Qualitative tools can aid in gaining a deeper understanding of behavioural risk activities by asking open questions and listening to participants' stories without predetermined ideas. This is the first study comparing environmental and socioecological distinct districts in Uganda through focus group discussions (FGDs) and key informant interviews (KIIs), to understand participants' perspectives on animal-human-tick interactions. The results provided information on additional questions to incorporate later to quantify risk for exposure to CCHFV. The qualitative locally-informed additional questions were next incorporated into the questionnaire of the serosurvey described further in Chapter 5.

The main objective of this chapter was to investigate local and cultural differences associated with human-animal-tick interactions in six socioecologically and environmentally distinct districts of Uganda, addressing the 2nd aim within the thesis outline.

The study sites were selected using K-prototype analysis, as described in Chapter 3, which combined environmental and socioecological variables to identify distinct clusters within Uganda. To gain an insight into human-animal-tick interactions at the study sites, KIIs and FGDs were conducted with participant groups, varying in sex, age and occupation.

Specific objectives to highlight relevant transmission routes of CCHFV through

ticks and direct contact with infected animal products were:

- **2.1** To identify the perceived burden of ticks and tick bites in each district and to highlight differences in tick bite risk.
- **2.2** To highlight interactions with ticks which could expose humans to CCHFV.
- **2.3** To analyse and understand slaughtering locations and methods, to understand the risk of direct transmission.
- **2.4** To identify differences between animal product consumption practices.

4.4 Methods

Ethical considerations and permissions

The AVQ study, short for 'ArboViral Qualitative', falls under the umbrella of the wider ArboViral Infection study (AVI study), which was approved by the Uganda Virus Research Institute Research Ethics Committee (UVRI REC) (GC/127/18/09/662) (Appendix B (p. 210)) and by the Uganda National Council for Science and Technology (UNCST) under the number HS 2485 (Appendix B (p. 215 and p. 217)). An amendment containing the details of this study was approved on the 7th February 2023 (Appendix B (p. 212)). The study was introduced and explained to the District Health Officer (DHO) and other district officials, where appropriate, and approval was sought to conduct the study within the district and local communities.

Informed consent was obtained from all study participants. An information sheet in the local languages, containing the necessary details about the study, ethics, and data usage (Appendix C (p. 219)) was shared, read aloud, and

discussed in the group, giving people time to ask questions and have them addressed. All forms were available in English and the district's main local language. This meant that for the recruitment in Soroti, the consent forms were available in Ateso, for Kasese in Lukhonzho, for Kaabong in Nga'Karimojong, for Kalangala and Kampala in Luganda, and for Arua in Lugbarati. A signature to consent to the study was recorded from every participant. In case of illiteracy, a witness, separate from the study team, joined the reading and discussion of the information sheet, signed the consent form, and the participant gave a thumbprint. For teenagers, one parent/legal guardian signed the official parental/legal guardian consent form document, which was either explained and read to them the day before by a local helper in the district, or the parent/legal guardian joined on the day of the FGD and left after the signatures took place. Teenagers were asked to sign or thumbprint an assent form if they agreed to join the study in keeping with UVRI REC recommendations.

Data management and confidentiality

Consent forms were filled out in duplicate, with one kept by the participant and the other by the study team. Copies were archived in a locked room at UVRI, and scanned and saved on a password-protected data stick, which was stored in a locked cabinet within a secure office room.

Every participant was given a study number, which was used to record demographic data on the secure platform REDCap (Harris *et al.* 2009). Additionally, participants in the FGDs were assigned numbers to avoid using their names during the discussions. No identifiers were added to quotes to ensure participants remained anonymous.

All conversations were audio-recorded and then translated and transcribed for analysis. Recordings and transcripts were stored on OneDrive, managed by the University of Glasgow.

Study design and data generation

This was a cross-sectional qualitative study, conducted in six environmentally and socioecologically distinct districts of Uganda. Site selection is described extensively in Chapter 3.

We conducted a total of 24 FGDs and 31 KIIs across the six districts, starting in November 2023 with Kampala, Kalangala, Kasese, Arua and Soroti, and Kaabong district was added in March 2024. This included four FGDs in each district with the following characteristics:
- a group of community leaders, including local councils, religious leaders, and women's leaders
- a group of men between 18 and 30 years old
- a group of women between 30 and 60 years old
- a group of teenagers around the age of thirteen, with mixed sex and varied educational levels

The reasoning behind the age groups in the men's and women's FGDs was that we realised in the Ugandan setting, the group of community leaders would encompass more men than women, and the overall age of these men would be somewhat older. Therefore, we decided to recruit younger men in the men's FGDs, and to have a different age group for women, we decided on the older age group for the women's FGDs to ensure wide representation.

The village centres, where the FGDs were conducted, were selected by the social scientist in the study, based on ease of access and previous experience of working with the community. In Kampala, the capital and largest city of Uganda, we focused on only one of the five divisions, the Kawempe division. The selection of participants was undertaken by helpers within the village centres, after explaining the study aims and the broad categories into which participants should be categorised, including age, sex, and occupation. All interviews were conducted within the larger town/city area of the district,

due to time and transportation constraints. They were spread geographically as far as possible, aiming to cover different insights and opinions around the towns/cities.

Five KIIs were conducted in each district, with purposively selected participants: a medical doctor, a veterinarian, the district surveillance focal person (public health specialist overseeing all public health surveillance activities for the district), a herdsman, and a traditional healer or herbalist. In Kampala, an extra KII was added with a meat inspector from Kalerwe abattoir.

Due to the different local languages in the districts, I worked with six social scientists who spoke the representative languages, including English, to conduct the KIIs and guide the FGDs in the languages preferred by the study participants (see Table 4.2). Many KIIs were conducted in English by preference, and in these cases, I was able to personally interview participants. All others were conducted by the social scientists.

Social Scientist	**District**	**Language**
Lazaaro Mujumbusi	Kampala	Luganda
Richard Muhumuza	Kalangala	Luganda
Mathias Akugizibwe	Kasese	Lukhonzho
Titus Apangu	Arua	Lugbarati
Edward Obicho	Soroti	Ateso
Evalyne Umo	Kaabong	Nga'Karimojong

Table 4.2: **Social scientists working on the AVQ study.** All social scientists were able to speak the local language as their mother tongue.

Demographic data of all participants in the FGDs were recorded in REDCap (Harris *et al.* 2009), (Appendix D (p. 225)). To guide the discussions and the interviews, a topic guide was created (Appendix D (p. 225)). This was split into five parts, including (1) an icebreaker, which included the demographics

and a question about the living environment of the people; (2) questions about the knowledge and understanding of viral haemorrhagic fever viruses (VHFVs), CCHFV, and ticks; (3) the social scientist gave general information about the above to the participants, and the participants could ask questions; (4) questions about behaviours and perceptions about transmission risks through tick bites and direct contact were discussed; and (5) in the final part, recommendations and comments from the participants were recorded, regarding CCHFV and tick control. The topic guides for KIIs were very similar to the topic guide of the FGDs, only including a few more guided questions relating to the occupations of the interviewed participants.

As described above, all FGDs and KIIs were recorded on a tape recorder, and translated and transcribed by the respective social scientists as shown in Table 4.2.

Data analysis

To analyse the data, I created a code book, including a coding frame with detailed explanations for each code, in partnership with Lazaaro Mujumbusi (LM). We each read through four transcripts (women's FGD Arua, community leaders' FGD Kalangala, one KII from Kampala, and men's FGD Kaabongs) to identify parent and child codes. Through joint discussions, we created one coherent coding frame and code book document, as shown in Appendix D.

All further coding was conducted using NVIVO (Lumivero, 2023), a platform that enables the coding of multiple transcripts and sharing between researchers. Manual coding was performed by three researchers, Richard Muhumuza, Mathias Akugizibwe, and me. This followed a thorough discussion of the codebook to harmonise the ideas and codes. Each researcher coded approximately one-third of all transcripts. The work was combined into a single project on NVivo and extracted using a simple script in R (R Core Team, 2021) into a large Mi-

crosoft Excel document (Microsoft Corporation, 2021), where all transcripts are columns and the paragraphs for each code from the codebook are ordered in rows. The R script is presented in Appendix A (p. 205).

Iterative categorisation (IC) was used to analyse the individual codes, to generate themes within codes and to prioritise themes to include in the results. IC was first described by Neale, 2016, and presents a flexible but structured approach to analyse and summarise qualitative data. Codes which include topics describing possible risk of transmission to CCHFV are included in the initial IC work, including, for example, codes on tick bites, slaughtering, animal contact or animal products. Further analyses included summarising all codes and focusing the analysis on the risk of transmission to CCHFV in each of the six study districts.

4.5 Results

Characteristics of study population

A total of 152 participants took part in the 24 FGDs and 31 KIIs across the six study sites described in Chapter 3. The median age of all participants was 36 years, ranging from 12 to 86 years (Figure 4.1). The women's FGDs had a median age of 45 years, while the men's FGDs had a median age of 30 years (Figure 4.1). Overall, more men participated than women (61% vs 39%) (Figure 4.2). This is explained by the overall trend in mixed groups, including the teenage and community FGDs, which contained more men than women on average. However, the strongest sex difference (87% men) was observed in the KIIs, which included a high proportion of men who were district personnel. The recruitment of participants purposely included Christians and Muslims, the two predominant religions in Uganda. Various occupations were reported; however, the dominating groups were farmers and businesspeople.

Most participants in the KIIs studied at Universities (including medical doctors, veterinarians, and district surveillance focal persons). Within the FGDs only a few participants had any form of tertiary education, around half had attended secondary school, and the other half left formal education during or at the end of primary school, with a few who had never received any formal education.

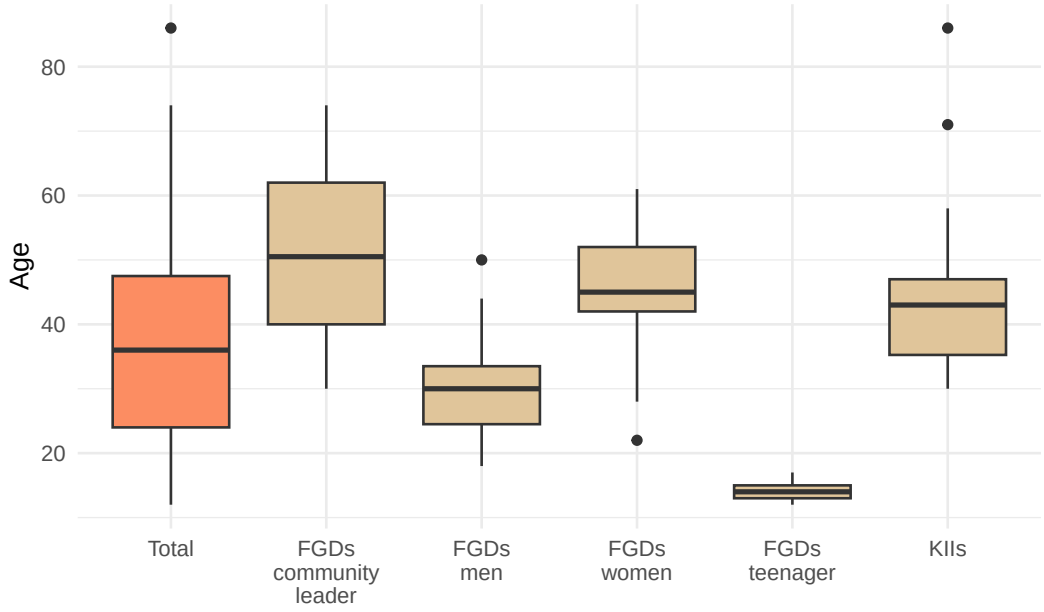

Figure 4.1: **Age distribution within focus group discussions (FGDs) and key informant interviews (KIIs).** The total age distribution and the distribution within each group are represented in box plots. Ten participants did not provide their age and were excluded from the graph.

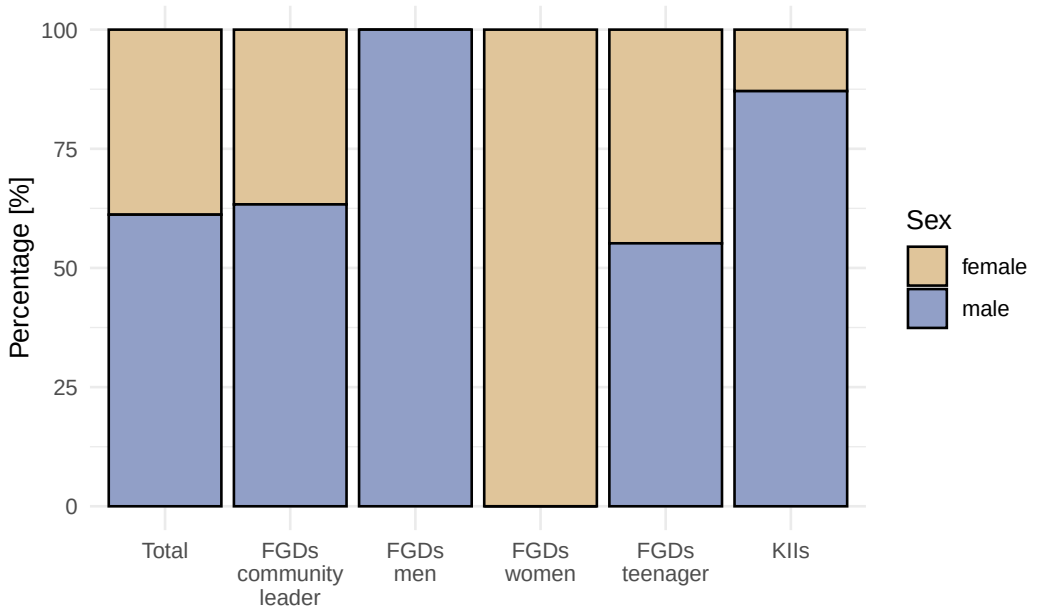

Figure 4.2: **Sex distribution within focus group discussions (FGDs) and key informant interviews (KIIs).** The total sex distribution and the distribution within each group are represented in stacked bars.

Analysing different transmission routes for CCHFV

In the FGDs and KIIs, different practices and events were described across the six districts, which could put people at risk of CCHFV infection. Several aspects were discussed, and four key categories arose from the analyses. These were discussed at varying points throughout the FGDs and KIIs, but are presented here separately, ordered by the likely importance that such a behaviour might have on CCHFV infection risk. These four main categories were: risk of tick bites, tick consumption, direct contact during slaughtering, and animal product consumption.

1. Risk of tick bites

Tick bites were reported by participants in all districts. However, they were reported more commonly in some districts (Kaabong, Soroti, Arua, and Kas-

ese) than others (Kalangala and Kampala). This is also similar to participant accounts of tick sightings in the environment and on domestic animals. Even when not everyone notices ticks as regularly as this participant in the men's FGD: *"Ticks are common in my area (Soroti). It is normal to see a tick."*, ticks were reported in all districts. An example is a story in Kalangala, where when the focus of discussion centred on finding ticks, a participant's grandmother directly found one:

> *"My grandmother was digging, and we had just studied about ticks, and I asked her. Have you ever seen ticks? She replied that before you joined us here, we used to have ticks but learned to spray them. We were still digging when she said you are the one who has been asking about ticks. Come and see it and I saw it." (Teenage FGD Kalangala)*

In Kaabong, ticks are so common that multiple participants in FGDs and KIIs reported ticks as being part of the ecosystem. For example, as described by a participant in the men's FGD: *"Ticks live with us in the same community, they are part of us"*. As mentioned earlier, the abundance of ticks described by the participants tended to be reflected by the number of reported tick bites within the community. An example from the same participant in the men's FGD in Kaabong, who reported that *"ticks bite us every day and oftentimes we have just accepted to leave by them"*.

A notable situation happened while conducting the men's FGD in Kasese district, where the participants found ticks just below them in the grass: *"Actually, you may find a tick here because goats do graze here [points to the grass]. Yeah, it's even here"*. Three ticks were found in ankle-high grass under a tree shade during the FGD (Figure 4.3). This observation highlighted the most common answer to where ticks were found in the environment, which was where animals graze, feed, drink, or rest. Participants also described com-

monly seeing ticks in high grasses and bushes. Additionally, in Kaabong and Kasese, both areas with large national parks and wild animals nearby, participants mentioned that ticks are commonly seen there and that there is a risk of getting tick bites when entering parks to collect firewood or to poach wild animals. These were also reported to present a risk to domestic animals, which share water sources with wild animals:

> *"Let me talk of them also being so common in the bushes, this is because they feed on wild animals. To add on that I can say ticks are also so common around water sources where both domestic and wild animals go to water and graze from." (KII Kaabong)*

Across all districts, it was stated that children and young adults up to about 25 years old are most susceptible to getting bitten by ticks. This was explained in various ways:

> *"The young ones that play around the compound, playing with the dogs and goats, [are most susceptible to tick bites.]" (Women FGD Kalangala)*

> *"I wanted to say that those at the age of 10 plus [years are most susceptible to tick bites,] because some of these children even play with the ticks. When they see it on a cow, they pick it and put it on their skin to feel how it bites." (Community leader FGD Kalangala)*

> *"Just like the way my colleagues have discussed, the age groups that are most affected [by tick bites] are those boys of 6-15 and men of 16-25 years. This is because right from our great parents, it has been boys and men who take care of the animals, and also they are*

a)

b)

Figure 4.3: **Ticks found at the men's FGD in Kasese District.** a) Three ticks were picked up by the men during their FGD. b) Tick spotted in the grass on the same ground, likely seeking a nearby host to latch on. Both pictures were taken in October 2023.

the ones who spend much of their time taking care of the animals."
(Men FGD Kaabong)

Multiple reasons were stated as to why children and young adults are considered the most susceptible age groups to tick bites, and the majority related to contact with animals. However, when activities with animals were discussed, which included bringing animals for grazing and accessing water, cleaning resting places, feeding, and daily milking, sex also played a role in some FGDs. In the above quote from Kaabong, it was boys and young men who were reported to be looking after the animals. Contrary to that, in the Women's FGDs, it was often mentioned that women also play a large part in the activities with the domestic animals. This was especially discussed in the Women's FGD in Kalangala and Kaabong.

"It is a good thing you invited us the women because we are the ones that do that work (caring for animals). Even if it is milking. I milk my own cow. Even taking it to the bushes to eat I take it."
(Women FGD Kalangala)

Kalangala, Kampala and Kasese District were three research settings where the risk of tick bites was also mentioned for adults and older people, either as a statement that all people, regardless of their age, could get a tick bite, or specifically mentioning older people.

A common theme across discussions at all study sites was that domestic animals commonly harbour ticks. A participant of the KIIs in Kalangala said: *"I've never gone to a farm, and I didn't see a tick, [] on an animal"*. Multiple reasons were discussed for why people keep animals, and which animals they keep. The main reason for most was the consumption of milk and meat from animals, which will be discussed later in detail as a risk of direct transmission of CCHFV. But there were also other reasons why people owned animals. One

is the status in the community and linkage with power and wealth. Animal gifts to the family of the son's bride as dowries are considered *"cultural payments"* (Women FGD Arua), and are required for weddings. Dogs and cats are kept for security reasons (Figure 4.4), pest elimination, hunting wild animals, as well as for petting in districts like Kampala, Kalangala, and Soroti.

> *"The animal is a precious bank for the communities. It is even a source of prestige because when you have animals or a herd of animals, you are a social capitalist. You have power to make decisions on affairs of the community because you will be contributing to the community. When there is a funeral, you will be looked at as a savior." (KII Arua)*

> *"We have cats for security and [they] are believed to detect witchcraft. They are treated as family members" (Community leader FGD Kalangala)*

The reasons why people own animals might change their behaviour towards different types of animals, and how animals are kept at night. Large herds of animals are kept in kraals, which are enclosed spaces for animals to find shelter. Kraals were reported in all districts, but were less common in Kasese. Animals, especially goats and chickens, were commonly reported to be kept in the main house at night in Kasese and Arua. This practice was also mentioned less frequently in the other districts, except Soroti. The main reason cited was the insecurity at night and the fear of theft. Additional reasons were a lack of other options due to confinement or financial means. Keeping animals in the house can mean close contact over a long period, where ticks can move from animals to humans.

> *"We also have a norm in Bakonjo that says whether a man or a woman shouldn't miss having a goat in his or her home. [] And*

Figure 4.4: **A cat called Mayanja**, who was believed to protect his owner from bad people. Mayanja sat in the tree just above the bench where the interview of a KII took place in Kalangala. When the owner called him and told him that we, the visitors, were nice people, he miaowed quietly, slowly came down and was petted by the owner. The picture was taken in October 2023.

you don't have where to Keep it so you end up putting it in your house." (Community leader FGD Kasese)

Peridomestic animals were also mentioned as sources of ticks by participants. Rodents were reported by a participant in the teenage FGD in Kaabong to be *"ever surrounded by ticks all over their bodies"*. Rodents are common around houses in all study sites, can carry ticks, and get very close to humans, especially at night, as highlighted by this participant reflection:

"You will come to notice the presence of rats when your hands and feet may be eaten up by rats at night and you will come to notice this with pain while washing your hands. When you check the hand or feet, rat teeth marks will be evident." (Men FGD Arua)

To summarise, participants associated the risk of tick bites with particular kinds of areas, groups, and activities. Across all six settings, children were seen to be particularly vulnerable to tick bites because of both their social roles (herding cattle, helping with milking) and their curiosity and propensity to play with animals. Overall, participants highlighted the importance of the presence of animals in the risk of tick bites.

2. Tick contact

Ticks are regularly removed from animals by hand or by spraying affected animals with an acaricide to kill the ticks on the animals. Regarding the risk of contact and potential transmission of CCHFV from ticks and animals to humans, one behaviour stood out, which was previously highlighted in a quantitative survey by Atim *et al.* 2022 but which has never been investigated in detail in a qualitative study before. This was the consumption of engorged ticks plucked from animals and then roasted in an open fire. Participants reported that ticks were eaten either for their protein value or that the people

roasting and eating them see this as a punishment for sucking blood from their animals. In Kaabong, this was reported as a practice from the past and is no longer conducted, with one herdsman explaining the reason as: *"Our generation is full of a lot of diseases, that is why ticks are no longer edible"*.

In contrast, in Arua, this practice was found to be commonly conducted and was well known. We observed the preparation of a tick by several herdsmen near Arua city (Figure 4.5). Many participants reported seeing this practice, but it was often not reported as being enacted at the time by participants in Arua, suggesting that trends may be moving in Arua towards non-consumption as observed in other districts.

> *"As they eat the ticks, they make comments like "these are the ticks sucking blood of our animals and we must punish them." (Women FGD Arua)*

> *"When we were looking after animals some years back, when I was around fourteen, thirteen, we also used to eat ticks." (KII Arua)*

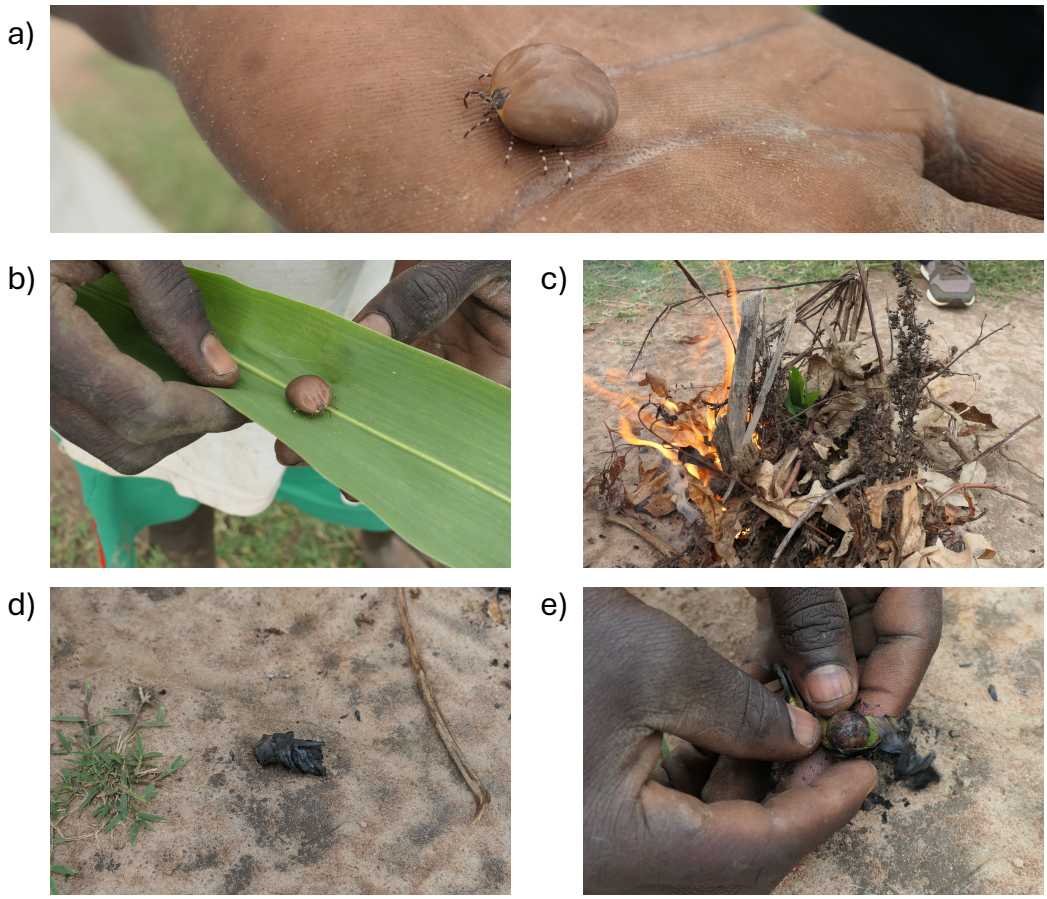

Figure 4.5: **Roasting an *Amblyomma variegatum* tick in Arua.** a) The tick was extracted while fully engorged from a cow. b) The tick was next wrapped in a leaf. c) A small fire was lit, and the leaf-wrapped tick was thrown in the middle. d) The leaf-wrapped tick was removed from the fire when fully blackened, after around 5 minutes. e) The roasted tick was removed and consumed. All pictures were taken in November 2023.

3. Direct contact during slaughtering

Slaughtering was another key activity performed in close contact with animals, providing direct contact with animals as well as an opportunity for ticks to crawl from animals to humans. The risk of direct transmission of CCHFV, through contact with the body fluids of an infected animal, was described in the men's FGD in Kampala: *"You cannot slaughter a cow or goat or even a chicken without getting into contact with its blood"*. Most participants in all research settings mentioned that they had slaughtered themselves before, or that they knew family members or community members who had carried out this activity. Slaughtering was most often reported to take place at home or at the farm, rather than at an abattoir. There were multiple reasons described for this:

> *"They cannot transport the cow on the boat to bring it to the slaughterhouse and take the meat back home. So what they do is slaughter from their homes and that is it." (Community leader FGD Kalangala)*

> *"What we have in Kalangala is the abattoir, but it is for only cows. For the goats, they slaughter them from anywhere, and the same applies to pigs." (Community leader FGD Kalangala)*

> *"When one dies, they perform rituals of killing an animal during the burial, this is like cleansing the whole family from the death." (KII Kaabong)*

> *"Because we have the Muslim community, they come and buy, and everyone wants to sacrifice, the goat or sheep or a cow to recognise the ritual of our grandfather Ibrahim." (KII Kampala)*

"We tend in Teso to make sacrifices such as sacrificing a white sheep, a black goat, sometimes, at a family level, we agree to remove some witchcrafts that were brought by our forefathers." (Community leader FGD Soroti)

The remoteness of places such as the islands of Kalangala, the lack of official abattoirs, rituals for events in life like marriage, birth and death, and religious rituals are all reasons mentioned for slaughtering animals at participants' homes. Abattoirs were only mentioned as a means to sell the meat to the public.

When animals are slaughtered privately, it is unlikely that there is any veterinary personnel who inspect the animal pre- and post-mortem. This may then lead to, or enable, the slaughtering of sick animals or butchering and consuming animals which died from a natural death. Participants in all districts, except Kampala and Arua, mentioned the slaughtering of sick and dead animals for consumption. Various reasons were mentioned, including the commercial value of the animal, its nutritional value, or ignorance. In Kaabong, the slaughter of sick animals is widespread and even the norm, as mentioned by participants in the women's FGD:

"Here in my community, we hardly slaughter animals for consumption unless if it has been sick and it dies." (Women FGD Kaabong)

"If my cow is sick; do you think I will bring it here [Kalangala town council to the abattoir]? We just slaughter it from the farm, share the meat and call it a day." (Community leader FGD Kalangala)

There are different methods of how animals are slaughtered, explained by a participant from the KIIs in Kasese: *"For us, every animal has its own way of slaughtering it"*. In Kampala, Kasese, Kalangala and Soroti, it was mentioned

that they cut the neck of the animal to kill it. Kaabong was the only place they mentioned where they pierce the animals' necks:

> *"In my community, animals are slaughtered through piercing the animal's neck and blood is picked and taken, then after it is skinned" (Teenage FGD Kaabong)*

Different slaughtering methods might increase or decrease the risk of coming into contact with infectious body fluids, and these methods likely differ due to the intended use of the animal and its products. As the above quote suggests, the neck piercing method is standard in Kaabong, as blood is regularly consumed there, which is further described in the following section as another potential exposure route of interest for CCHFV.

4. Animal product consumption

Consuming meat and other animal products from previously infected animals may be a lower risk transmission route for CCHFV. However, a few practices stood out during the interviews and discussions, highlighting the possibility of transmission of CCHFV infection, through consuming sick animals or wild animals, as well as eating uncooked, or only half-cooked, products.

As described earlier, participants described multiple reasons why sick animals were slaughtered and later consumed. A participant highlighted in the women's FGD in Kasese that: *"You cannot throw away meat. That is meat. Meat remains meat. I cannot throw my cow"*. This highlights the focus on the value of meat, rather than possible infection risks through consumption.

Wild animals, including antelopes, wild birds, wild large rats, and others, were described as being hunted by members of the communities. These were reported to be infested with ticks and may be associated with a risk of transmission of known and unknown infections. These species have an unknown

risk of harbouring CCHFV infection. In Kaabong, multiple participants mentioned the consumption of wild rats, and in Soroti, a participant mentioned hunting birds.

> *"Are you aware that we eat or feed on rodents, we hunt them down from the bush using traps and consume them and this is one of the best meat around." (Teenage FGD Kaabong)*

> *"There is a tendency of young boys having catapults. They were looking after cows at the same time hunting birds down." (KII Soroti)*

Reports of meat being eaten half-cooked or even raw were common in several districts. Consuming half-cooked meat was reported when animals might be roasted as a whole and middle parts might not be completely cooked, when animals were roasted in the forests or shrines for ritual, or when there was a sense of urgency, for example when *"like around 20 people are waiting so you have to be fast enough to meet the demand [of roasted pork at a place of excitement like a bar]"* (Community leader FGD Kalangala).

In addition to eating meat, consuming animal blood was commonly reported by multiple participants in Kasese, Kaabong, Arua and Soroti Districts. However, there were differences in the preparation and the means of consumption. In Kasese, participants discussed rearing guinea pigs, which are slaughtered for the primary purpose of drinking their fresh, uncooked blood to fight anaemia. In Arua, a blood meal is cooked in advance, and holds a strong meaning given the name *"culture"* after preparation, as mentioned by multiple participants. Similarly, in Kaabong, where raw blood is consumed uncooked, it is closely linked to ceremonies and special occasions like weddings or to prepare for an animal raid in neighbouring villages. It was also seen as a nutritional meal, similar to reports in Kasese.

"You just cut it (the guinea pig) and take its fresh blood fighting anemia." (Community leader FGD Kasese)

"In addition [to preparing the blood], they also squeeze the faeces (fresh dung) which is still in the animal's stomach and mix with blood as they cook." (Community leader FGD Arua)

"When they bled an animal on the neck and blood is extracted from it and it is then mixed with milk taken by the community members. It is one of the activities done in our community and it is part of us." (KII Kaabong)

Blood was also frequently mentioned during rituals, such as sacrifices, healing, funerals, and weddings. It may be poured or smeared on a person, people bathe in it, and it is consumed or "given to the ancestors". Similar stories were told about intestines, where elders in Kaabong use them to foresee the future.

Overall, a range of potential risk factors were mentioned relating to tick bites and tick presence, consumption of ticks, slaughtering and eating meat and other animal products and direct contact with healthy and sick animals and carcases. Some practices varied strongly by district, like tick consumption, which was only present in Kaabong and Arua, while others were widespread within the study populations in most districts, such as regular tick bites and close contact with animals.

4.6 Discussion

In this study, we used FGDs and KIIs to explore people's views on human-animal-tick interactions in six distinct districts in Uganda. The focus of this

study was to understand the transmission risk of CCHFV primarily through tick bites and tick consumption, direct contact with live animals and during slaughtering or consuming animal products, and to implement key observations into a future quantitative survey. In the FGDs and KIIs, participants described differences in perceived tick burden, which correlated with reported tick bite prevalence. The highest reported abundances occurred in the northern and western districts: Kaabong, Arua and Kasese. Historical consumption of engorged ticks was reported in Kaabong, while consumption was still practised in Arua District. Slaughtering at home and slaughtering sick animals was very common in all districts. The consumption of raw blood was limited to Kaabong and Kasese districts.

In our study, perception of tick burden and tick bites was heterogeneous, with the highest reports in Kaabong and Arua districts, and the lowest in Kampala and Kalangala districts. Ribeiro *et al.* 2023 conducted a study linking human tick bite risk to the tick abundance in the environment. The study investigated orienteers in Scotland, studying very specific activities and locations. Further similar studies in the study sites to understand the role of typical outdoor activities in the Ugandan setting could help to further explore the key findings in this chapter.

Reports about tick observations were mainly mentioned together with animals, either relating to animal contact or areas where animals eat, rest, or drink. Domestic and wild animals are known to be asymptomatic reservoirs for CCHFV (Hoogstraal, 1979; Spengler, Bergeron, & Rollin, 2016) and are commonly infested with ticks (Atim, Ashraf, *et al.* 2023; Balinandi *et al.* 2020; Khoule *et al.* 2025). This is in keeping with the observations of the participants in this study.

We did not clearly identify sex roles regarding activities with domestic animals. It was mentioned that mainly boys and young men looked after the animals in Kaabong district, but in the same district, women mentioned in their FGD

that they carried out feeding and cleaning of the animals in the kraals. In this work, we did not separate questions regarding small animal groups at the homestead from contact with larger herds for commercial purposes, as Agaba *et al.* 2025 discussed in their study. Agaba *et al.* 2025 presented a clear separation between the activities, very similar in all study sites, and future work would likely benefit from making this distinction clearer.

Participants in this study reported children as the highest risk group for tick bites. There is a paucity of scientific literature on the specific risk of tick bites by age group for CCHFV exposure. However, Lkhagvatseren *et al.* 2019 presented a discrepancy of high numbers of reported tick bites in children, with no clear evidence of higher exposure to tick-borne pathogens. However, Lyme disease surveillance in the USA reported the highest risk for children up to the age of 14 (Murphree Bacon *et al.* 1992). A similar study in Canada reported that children (5-9 years) and older adults (50-79 years) had the highest incidence rates (Adams *et al.* 2024).

Occupational risk relating to daily handling of animals was evident and several occupations are therefore likely to be at high risk for tick bites and CCHFV exposure, including herdsmen (included in the KIIs), abattoir workers, farmers, hunters/poachers and veterinary personnel. Other studies have shown that tick bites are common in certain professions, for example, in forest workers and farmers in Germany. However, such occupations are highly context-specific and further studies are required to delineate local risks in Uganda (Schielein *et al.* 2022).

The perceived risk of tick bites from wild animals in national parks has previously been shown by multiple studies collecting ticks from wildlife (Lacroux *et al.* 2023). Several wild animal species are well-reported to be exposed to CCHFV and have high seroprevalences in studied populations (Celina *et al.* 2024; Spengler, Estrada-Peña, *et al.* 2016), indicating that ticks which feed on wild animals are likely to be infectious. This is in keeping with the perceived

risk by the communities in this study.

Eating engorged ticks in Uganda was first mentioned in scientific literature by Atim *et al.* 2022 and was significantly correlated with higher exposure rates to CCHFV in Arua district (Atim *et al.* 2022). We have recorded for the first time that not only in Arua, but also in Kaabong, people have historically consumed engorged ticks. Eating engorged ticks has also been reported in other countries. A recent study in Cameroon reported that 3% of interviewees had eaten ticks (Gasparine *et al.* 2025). All records were relatively recent, and there is a likely possibility that other communities in other locations might practice the same. This should be investigated further in future studies.

While all participants mentioned that ticks were roasted before consumption, there is a chance that ticks might be eaten before virus particles are completely denatured, as efficient heating is required to denature the viral particle (Saluzzo' *et al.* 1988). Conversely, it is possible that sometimes viral proteins are sufficiently denatured and could hypothetically act as a mucosal vaccine against CCHFV infection. This, alongside exposure to other nairoviruses, known to occur commonly in ticks in Uganda, might in fact provide a protective effect. Further investigation of this hypothesis is required and will be carried out as part of a new study carried out by the Thomson group, funded by EEID for 5M US dollars.

A very high-risk route of infection is likely to occur while plucking ticks from animals with bare hands. Clearly, blood could enter through wounds or microcuts/abrasions, allowing the virus to enter through the skin barrier. Direct transmission from CCHFV patients' blood or body fluids in hospital settings to healthcare workers through skin contact has been well-described, highlighting that this is likely a high-risk activity (Gozel *et al.* 2013).

A similar exposure route is expected when slaughtering infected animals or consuming infectious animal products. CCHFV can enter through microcuts

in the skin or mucosa when a virus particle from blood or body fluids comes into contact with the individual. Slaughtering may be likely to be associated with particularly high exposure. Infections through food intake have also been reported from uncooked meat (Fazlalipour *et al.* 2016; Sharifi Mood *et al.* 2011), and abattoir workers have a high risk of exposure to CCHFV (Akuffo *et al.* 2016; Sheek-Hussein *et al.* 2025).

The common reports of slaughtering at home by multiple participants open the possibility of high exposure in Ugandan communities, as some individuals might have less training, expertise and experience than professional abattoir workers. Evidently, for home slaughters, there would not be a supervising veterinary officer present to check on the animals pre- and post-slaughtering, to identify sickness and prohibit activities if necessary. This was one of the reasons mentioned by the participants regarding why they slaughtered from home, to avoid such controls and be able to slaughter sick animals, and not to lose precious meat sources. Slaughtering and eating meat from sick animals has been reported in other countries, for example, in a study in Kenya where 9% of slaughterhouses slaughtered and sold sick animals (Cook *et al.* 2017), and in a Nigerian study where 55% of participants believed that diseases could not be contracted from eating sick animals (Sylvia *et al.* 2024).

While the main and recurrent themes associated with exposure to ticks and animals were selected for discussion in this chapter, other associations with risk, in addition to those reported above, should be considered further in the future. All transcripts were coded and will be made available for researchers as an anonymised dataset in Enlighten following the publication of this work.

This study was designed to capture a variety of behaviours and differences across Uganda, using K-prototype analysis to select heterogeneous districts (as described in Chapter 3). Several high-risk activities were highlighted, and the heterogeneity of the FGDs and KIIs findings supports the care taken to locate geographically and culturally different areas of the country. However,

while these findings are important, the study could not fully represent the whole of Uganda and wider endemic areas for CCHFV. Recruitment was opportunistic within selected districts, based on the availability of participants and ease of access. In the field of local ecological knowledge (LEK), researchers have discussed identifying experts within communities, and state that rigorous reporting and a more systematic approach, such as identification by peers or systematic surveys, could help document knowledge more thoroughly (A. Davis & Wagner, 2003). For future work on CCHFV, this would especially apply to methods of controlling tick burden in the communities, as these data would benefit from more rigorous documentation.

While the selection of social scientists who spoke the local languages fluently in this study was a definite advantage in the study, the use of multiple different researchers conducting KIIs and FGDs may have somewhat biased reporting on differences when questions were asked in a slightly different style. A meeting with everyone present to discuss the setup in detail would have benefited the study, and it will be considered for future work.

This study added valuable insights for the quantitative serosurvey presented in Chapter 5. During recruitment and the start of data analysis, the information was used to improve the survey questions with local insights into behaviours which could be risk factors for the exposure to CCHFV. This included questions regarding contact with wild animals and wild birds, more detailed questions around the consumption of engorged ticks, and more details regarding activities with domestic animals.

4.7 Conclusions

This study highlighted extremely close human-animal-tick contact across six diverse Ugandan districts, with practices such as daily tick exposure, animal

cohabitation, and slaughtering of sick or dead animals, which are highly likely to contribute to CCHFV transmission risk. Specific and unique behaviours, including roasting engorged ticks and consuming fresh animal blood, underscore the need to quantify the frequency of these practices further and assess the actual level of risk they may pose. In part, we aimed to do this as part of a detailed quantitative study, described in Chapter 5. Such insights are essential for informing contextually appropriate public health strategies. These findings also reinforce the critical importance of integrating qualitative perspectives into epidemiological research.

Chapter 5

Sero-epidemiological study to understand risk factors and transmission of Crimean-Congo haemorrhagic fever virus in Uganda

5.1 Abstract

Crimean-Congo haemorrhagic fever virus (CCHFV) is a tick-borne zoonotic pathogen causing fever and non-specific symptoms that can progress to a severe haemorrhagic disease, with up to 31% fatality among hospitalised cases. Uganda has seen a rise in reported infections over the past decade, though mild or misdiagnosed presentations limit accuracy, leading to an underestimation of true case numbers. Local environmental and socioecological risk factors are poorly understood, and high-risk populations have not been investigated in detail prior to this PhD. A deeper understanding of socioecological risk factors

is crucial for guiding effective interventions.

In this chapter, an analytical framework was developed to estimate seroprevalence in four distinct districts of Uganda as part of an interim analysis of the wider ArboViral Infection study (AVI study). Sites were selected through K-prototype analysis. 320 participants were recruited through multi-level randomisation and stratified by age in each district. Serum samples were collected from each participant, and a structured survey was performed, which was informed by qualitative research. CCHFV antibody testing was carried out to estimate CCHFV exposure.

Data analysis highlighted varying estimated seroprevalence to CCHFV, ranging from 2.2% in Kaabong district to 18.2% in Kasese district. A multivariable analysis, including known risk factors for CCHFV transmission, revealed significant differences in CCHFV seropositivity between study locations and age groups, with more minor effects of behavioural factors such as reported tick bites. The force of infection (FOI) showed an accumulation of seropositivity with age, suggesting constant exposure.

The strongest indicator for seropositivity was the study locations, highlighting the importance of environmental factors for CCHFV transmission in Uganda. This information has the potential to identify high-risk regions within Uganda and guide control strategies for CCHFV transmission, including the implementation of tick control or vaccine trials in high-risk areas.

5.2 Acknowledgements

This study involved many groups and people. Complete acknowledgement is presented in Table 5.1.

This study was funded by my personal grant as part of the Wellcome Trust

	Contributions
Marina Kugler	Setting up of the study, including ethics, study planning, protocols, and survey development Leading recruitment in Kasese, Soroti and Kaabong districts, and overseeing recruitment in Kalangala CCHFV assay in Glasgow and UVRI Analysing, creating figures and tables
Stella A Atim, Shirin Ashraf, Leah Owen, Chris Davis, Emma C Thomson, Paul C D Johnson, Kimberly Fornace	Study oversight and design
Tomáš Janoušek	Helping with the random number generator
Joshua Muhindo, Edson Masereka, Laheri Kabugho, Merecy Musoki, Eliud Mumbere	Recruitment in Kasese
David Onanyang, Stephen Oumo, Hajjat Akiror, William Oriokot	Recruitment in Soroti
Don J B Lopoi, Lino Lokol, Esther A Awor, Emmanuel Lotyang, Gorety M Nalem	Recruitment in Kaabong
David Onanyang, Yofesi Nikweri, Livingstone Musoke, Gertrude Nansubuga, Rowan W Kafuuma, James Bugembe	Recruitment in Kalangala
Alex Tumusiime, Dianah Namanya, Stephen Balinandi	Help with CCHFV assay, and testing convalescent samples at UVRI

Table 5.1: Contributions to AVP study.

PhD funding (218518/Z/19/Z). Additionally, it was funded by the UK Medical Research Council (MRC) and the UK Foreign, Commonwealth & Development Office (FCDO) under the MRC/FCDO Concordat agreement and is carried out in the frame of the Global Health EDCTP3 Joint Undertaking (Preparedness Platform MC_UU_00034/6).

5.3 Introduction

Multiple opportunistic studies have investigated risk factors for Crimean-Congo haemorrhagic fever virus (CCHFV) infection and exposure in Uganda (Atim *et al.* 2022; Balinandi *et al.* 2022; Mirembe *et al.* 2021). However, before this study, no prior investigations were designed to investigate risk across the country systematically. In this chapter, I describe the first household-based, district-representative serosurvey which systematically investigates risk factors across ecologically diverse parts of the country, using a randomisation approach.

Chapter 4 highlighted socioecological behaviours which had not been investigated in a large serosurvey. This included the consumption of engorged ticks, hunting for wild birds, and using blood or animal products in rituals. These factors were added to refine the survey questionnaire design and were analysed alongside known risk factors for CCHFV exposure.

The main objective of this chapter was to estimate exposure to CCHFV in four districts across Uganda and analyse associated risk behaviours using a cross-sectional, household-based, randomised serosurvey, addressing the 3rd aim within the thesis outline. The study sites are a subset of the districts described in Chapter 3 and formed an interim analysis of a larger study. The work followed the qualitative study presented in Chapter 4, and included an evaluation of key socioecological behaviours identified in this work. In this

chapter, the following objectives were addressed:

- **3.1** To recruit participants in four environmentally and socioecologically distinct districts in Uganda, and assess their exposure to CCHFV through serological testing.
- **3.2** To estimate CCHFV exposure at district, village and household levels, and identify spatial trends or patterns.
- **3.3** To identify and analyse behavioural risk factors associated with CCHFV exposure using univariable and multivariable logistic regression models.

5.4 Methods

Ethical considerations and permissions

The seroprevalence study falls under the umbrella of the ArboViral Infection study (AVI study). The AVI study was approved by the Uganda Virus Research Institute Research Ethics Committee (UVRI REC) (GC/127/18/09/662) (Appendix B (p. 210)) and by the Uganda National Council for Science and Technology (UNCST) under the number HS 2485 (Appendix B (p. 215 and p. 217)). An amendment containing the details of this study was approved on the 7th February 2023 (Appendix B (p. 212)). The Commissioner for Integrated Epidemiology Surveillance and Public Health Emergencies in the Ugandan Ministry of Health supplied us with a support letter for the initial contacts in the district (Appendix B (p. 218)). The study was introduced and explained to the District Health Officer (DHO), the Chief Administrative Officer (CAO), the District Veterinary Officer (DVO), and others when needed, and approval was sought to conduct the study within each district.

Written informed consent was obtained from all study participants. For adults above 18, the data information sheet plus consent form is enclosed as Consent form D in Appendix C (p. 222). For all children, one parent or guardian signed a parental/ guardian Consent form J. Children between 8 and 17 signed an assent form, Consent form K, in addition to the official consent form J, which was signed by the parent or guardian. If the person could not read or write, the participant provided a fingerprint, with an independent witness present, who signed instead.

All consent forms were available in English and the district's primary local language. The varying primary local languages within the study districts meant that for the recruitment in Soroti, the consent forms were available in Ateso, for Kasese in Lukhonzho, for Kaabong in Nga'Karimojong, and for Kalangala in Luganda.

To enable the use of samples in the longer term for further projects related to infectious diseases, we also asked the participants to allow their serum samples to be stored in a biobank, for which another consent form was signed (Consent forms E and F, Appendix C (p. 224).

An optional rapid test for HIV was offered at recruitment, with onward referral to specialist services as guided by the national HIV/AIDS programme.

Data management and confidentiality

All survey data were collected and recorded on REDCap (Harris *et al.* 2009). REDCap is a secure web application that is also available as a mobile phone app. The app can be used offline, which enables recruitment without an internet connection. Due to the remoteness of some study villages, we worked offline on all phones and uploaded data every evening to the server.

Consent forms, which included study information sheets, were filled out in

duplicate, with one kept by the participant and the other by the study team. They were archived during recruitment and transported to the Uganda Virus Research Institute (UVRI) for long-term storage. Patient details and consent forms were scanned and saved on a password-protected data stick and stored in a locked cabinet within a secure clinical office room.

Study design

The study was a cross-sectional household cluster-based survey, with multiple levels including regions, districts, villages, and households. The study sites were selected using K-prototype analysis, as described in Chapter 3. This resulted in 13 distinct clusters and the selection of six distinct environmental and socioecological districts within Uganda (see Figure 3.5). This chapter describes an interim analysis of four out of the six districts, including Kasese, Soroti, Kaabong and Kalangala. Final recruitment of all six districts will be carried out as part of the wider MRC-funded AVI study. In each district, a study team was formed and trained to conduct the recruitment in the local languages within the communities.

Recruitment started in Kasese district in December 2023 but was halted due to insecurity in the area following Foreign, Commonwealth and Development Office (FCDO) advice. The Soroti District study site was fully recruited between February and March 2024. Recruitment in Kaabong District started in March 2024 and was completed in April 2024. Recruitment in Kalangala District was conducted and completed between October and December 2024.

Selection of study villages and households

We recruited from eight villages per district to balance the broad coverage of the whole district with enough participants by village to reach 320 per district (see below). A list of all villages in the districts was downloaded from

the Electoral Commission of Uganda, created in 2022 (Electoral Commission, 2022). The eight villages were selected randomly using the randomizr library in R 4.2.0 (Coppock, 2023; R Core Team, 2021). District study teams identified the location of randomly selected villages.

The second stage of the cluster randomised survey was carried out in the villages. Local village health teams (VHTs) recorded all households in their village. VHTs are trained personnel hired by the government of Uganda within each village to facilitate health care in rural areas. Upon the study team's arrival and first introductions, the VHTs generated an updated list of all households in the village. The total number of households was used to randomly select the households to be asked to participate in the study, using the same library in R. An A list was created to represent the first ten households to be included. B and C lists were created to supplement the A list if insufficient participant numbers were available following the first randomisation.

Selection of study participants

The original sample size calculation for the serosurvey was based on previously observed seroprevalence rates reported in Atim *et al.* 2022 and the healthcare workers (HCWs) study (Chapter 2). In this study, observed CCHFV seropositivity ranged between 13% and 38%. Employing a significance level of 0.05, and a statistical power exceeding 80%, a sample size of 250 participants per district was estimated to be sufficient to detect a difference between districts of $> 5\%$ in seroprevalence.

Subsequently, a more conservative analysis was conducted to account for the cluster-based survey design. A design effect was incorporated into the sample size calculation to adjust for clustering, using a value of 2, which is commonly applied in comparable household surveys when no prior intracluster correlation data are available (Bostoen & Chalabi, 2006). This adjustment increased

the required sample size to detect meaningful differences in seroprevalence between districts. Maintaining a power above 80% and aiming to detect a modest difference in seroprevalence, a revised optimal sample size of 320 participants per district was selected. This allowed for detecting a significant difference of approximately 14%, assuming a baseline prevalence of 20% or lower. When comparing broader regional groupings (by combining districts), statistical power increases and the minimum detectable difference decreases.

As Uganda is a young country, with 44% of Ugandans below the age of 14 (WHO, 2025a), we added age groups to the study design, to represent all ages within the population and to deliberately over-represent older participants in the study, to receive more information on risk behaviours from adults. We recruited a maximum of one person within each of the following four age groups per household:

2–14 years

15–27 years

28–40 years

41 years and above

Randomisation within the household was conducted in REDCap (Harris *et al.* 2009). We used the recorded longitude (long) and latitude (lat) for each household using the following equation (Equation 5.1) to randomly select one participant out of all people within the same age group of the household.

randomly selected individual in $[\text{age category}]_i =$

$$(round([\text{long}] * 100,000) * 7,187 + round([\text{lat}] * 100,000) * 5,689 + 6871)$$
$$- (rounddown(\frac{(round([\text{long}] * 100,000) * 7,187 + round([\text{lat}] * 100,000) * 5,689 + 6,871)}{[\text{number of individuals in } [\text{age category}]_i]})$$
$$* [\text{number of individuals in } [\text{age category}]_i]) + 1 \tag{5.1}$$

As not all age groups were present in all households, additional participants were recruited from further households (from newly randomised B and C lists). This was carried out to enable all age groups to be represented equally in the final dataset and increase the chances of identifying risk factors in less-represented age groups within households. Figure 5.1 illustrates a visual representation of the study design.

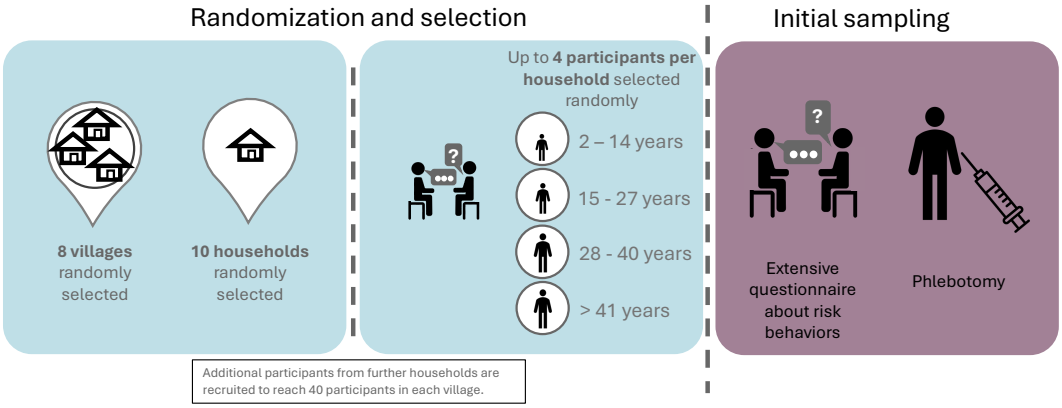

Figure 5.1: **Study design for the AVP study.**

Inclusion/Exclusion criteria

Inclusion and exclusion criteria are mentioned in Table 5.2. If fewer than 75% of the selected persons in a household were recruited, the whole household was excluded from the study. This ensured that the family not only agreed for certain household members to participate, and that the selection and participation remained random. Participants with a temperature of 38 °C or higher

were excluded from the study to lower the risk of recruitment of a participant with an acute infectious illness and to lower the biosecurity risk of the study.

Exclusion criteria	**Inclusion criteria**
- No consent - Not recruitable after two visits to the village - Children living away from home (e.g., full-time boarding school) - Less than 5kg - Anaemia - High temperature (38°C and above, measured by a temperature gun)	- Sleeps in the selected house most nights in the last weeks - Appears to be healthy (including no signs of anaemia, not significantly underweight)

Table 5.2: Exclusion and inclusion Criteria for study participants.

Data and data generation

The household survey recorded household characteristics, including the number of people in each age group, their location, and variables to calculate socioeconomic status, by interviewing the head of the household (Appendix D (p. 233)). The equity tool for Uganda was used to create the questions for socioeconomic status (Metrics for Management, 2022). This tool divides the country's population into quintiles of relative wealth for further analysis.

Further characteristics and risk factors for viral haemorrhagic fever virus (VHFV) exposure were recorded for each participant, using a structured questionnaire, translated by the study team in the local languages if needed (Appendix D (p. 237)).

A 10ml venous blood sample per adult was collected in a serum vacutainer collection tube (and a smaller weight-based sample was obtained from children), and stored on ice in a coolbox until the end of the day. In a designated laboratory located within each district, the samples were centrifuged at 2000g for 10min, and aliquoted into 2ml sterile storage vials (Sarstedt Inc, Newton, North Carolina). Serum was heat-inactivated at 56°C for 30 minutes and stored short-term at –20 °C within the district. After recruitment within the district was completed, samples were transferred on dry ice to UVRI and stored at –80 °C. One aliquot was shipped on dry ice to the MRC-University of Glasgow Centre for Virus Research (CVR) while two aliquots were retained at UVRI.

Serological assays

Several commercial enzyme-linked immunosorbent assays (ELISA) assays are available to test for antibodies against CCHFV in human serum. In our previous studies, we used VectoCrimean-CHF-IgG ELISA kits (VectorBest, Novosibirsk, Russia), which are based on whole virus antigen. However, as the kit was produced in Russia, it was no longer available in accordance with University and MRC guidelines after 2022. The ID Screen® CCHF Double Antigen Multi-species ELISA IgG (IDvet, Grabels, France) has been used in previous studies in Uganda for testing animal samples (Atim, Niebel, *et al.* 2023), and was recently validated for human use. Hughes *et al.* 2024 published a human seroprevalence study in Tanzania using this assay. Prior to commencing our serosurvey, we validated the ID Screen® alongside three other assays, using ten Ugandan convalescent samples from individuals with confirmed previous CCHFV infection, all of which were correctly identified as seropositive (Figure 5.2) . Further evaluation of ID Screen®, Anti-CCHFV ELISA from Euroimmun, and GP and NP CCHFV in-house ELISAs in Professor Teresa Lambes team are ongoing and will be reported by PhD candidate Dr Leah Owen.

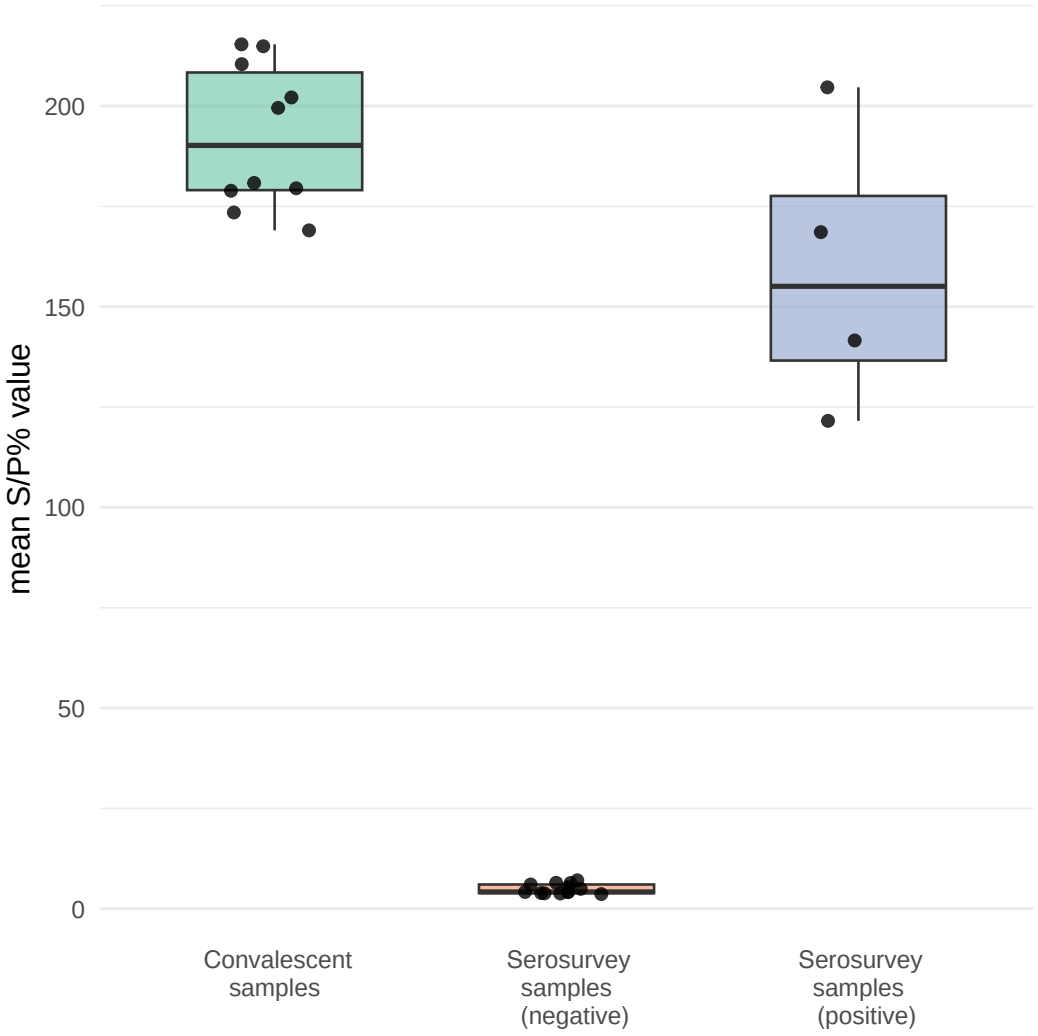

Figure 5.2: **Validation experiment for CCHFV serology.** 10 convalescent samples, and 17 samples (13 seronegative and 4 seropositive) were tested using the ID Screen® ELISA. The y-axis shows mean S/P% values (Sample OD/Positive control OD percentage). All convalescent samples tested positive.

Serum samples were tested in duplicate for specific antibodies against CCHFV using the ID Screen® CCHF Double Antigen Multi-species ELISA IgG (ID-vet, Grabels, France), following the manufacturer's protocol. The assay uses two antigen-antibody interactions to increase specificity (see Figure 5.3 for a visual explanation). Briefly, test sera were diluted (30µl serum + 50µl of manufacturer-provided dilution buffer) and incubated at room temperature for 45min. After five wash steps, 50µl of manufacturer-provided conjugate solution was added to each well and incubated for 30min at room temperature. Following five wash steps, 100µl of the manufacturer-provided substrate solution was added to each well and incubated for 15min at room temperature in the dark. The reaction was stopped by adding 100µl of manufacturer-provided stop solution, and absorbance was measured at 450nm. Each plate included a manufacturer-provided positive control, against which sample results were normalised (see Equation 5.2). Seropositivity was determined using the S/P (Sample OD/Positive control OD) percentage, with values above 30% considered positive.

$$\text{S/P\%} = \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{positive control}}} * 100 \tag{5.2}$$

Statistical analysis plan

Data were analysed using R 4.2.0 (R Core Team, 2021) and maps and graphs created with ggplot2 (Hadley Wickham, 2016). Tables were made and shown using the libraries table1 (Ren & Russell, 2021), formattable (Ren & Russell, 2021) and gtsummary (Sjoberg *et al.* 2021). In all analyses, variables that attained a p-value of < 0.05 were considered to be statistically significant. Copilot, an artificial intelligence tool by Microsoft (Copilot, 2025), was used to improve code and identify errors.

The survey design involved stratification by district and clustering at the vil-

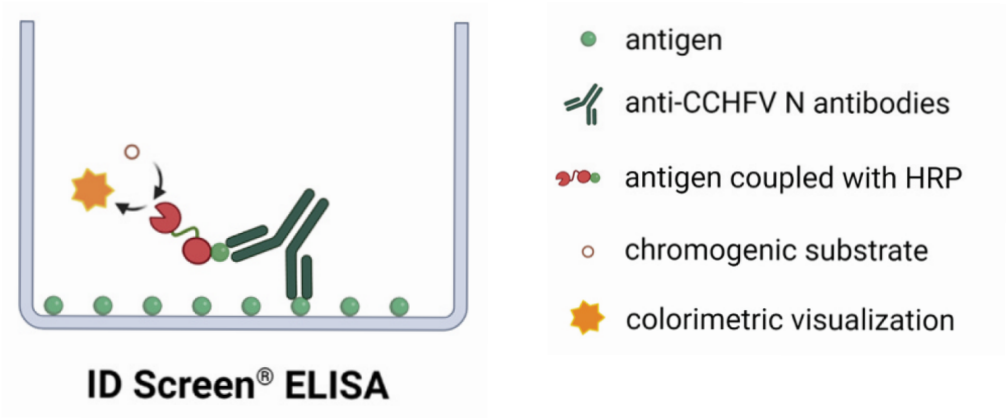

Figure 5.3: **IDScreen ELISA method.** Copied and adjusted from Bost *et al.* 2024.

lage and household level. I used functions from the survey library (Lumley, 2024) to account for this complex sampling design when estimating the seroprevalence for districts, villages and households. This allows population estimates and corrects standard errors. Sampling weights, which reflect the inverse of the probability (P) that the participant was selected at each stage, were calculated using Function 5.3 for the district estimates, Function 5.4 for village estimates, and Function 5.5 for household estimates of seropositivity for CCHFV. Only households from the initial list A (one from each age group from 10 households if available) were included in the estimation in order to prevent bias in households during later stages of recruitment.

$$\textbf{Weight (District estimate)} = \frac{1}{P(village_i) \times P(household_j) \times P(individual_k)}$$

$$= \frac{1}{\frac{\text{Villages sampled}}{\text{Total villages in district}} \times \frac{\text{Households sampled in village}_i}{\text{Total households in village}_i} \times \frac{1}{\text{Total n in age group of individual}_k}}$$

$$= \frac{\text{Total villages in district}}{\text{Villages sampled}} \times \frac{\text{Total households in village}_i}{\text{Households sampled in village}_i} \times \text{Total n in age group of individual}_k \tag{5.3}$$

$$\textbf{Weight (Village estimate)} = \frac{1}{P(household_j) \times P(individual_k)} \tag{5.4}$$

$$\textbf{Weight (Household estimate)} = \frac{1}{P(individual_k)} \tag{5.5}$$

I employed the function 'svymean' within the survey library to calculate the estimated mean for seropositivity within the districts. This function uses the Horvitz-Thompson estimation, as well as its variance calculation. It includes the probability of being sampled (calculated by the weights) and a probability for pairs to be joint, which is calculated by the specified stratification and clustering. To test for significant differences between the districts, the survey library has a predefined 'svyttest', which calls a generalised linear model (glm), corrected for complex survey design. This means that instead of the maximum likelihood estimations in a normal glm, it uses pseudo-likelihood numbers incorporating the study design, including weights for likelihoods and clustering and stratification for variance.

Village estimates were calculated using the same methods from the survey library. The centroids of recorded household GPS were used to plot the village seroprevalence using ggplot2 (Hadley Wickham, 2016). Outliers were excluded

due to expected errors in the GPS recording tool. The household size was calculated with the answers given by the participants on how many people were living in each household within each of the four age groups. These data were plotted for households in each village and by district in a heatmap using ggplot2 (Hadley Wickham, 2016). The household seroprevalence estimate for CCHFV could not be conducted using the survey library, as the numbers were too small. Instead, I calculated the weighted mean using the basic R function and weights as calculated in Function 5.5.

All participants were included in the risk analysis in order to identify risk factors for exposure to CCHFV. For all analyses, a generalised linear mixed model (glmm) was utilised to include random effects. Clustering within villages and households of all participants was corrected by including the household variable as a random effect in the model. This corrects for the correlations between individuals in a household, accounts for the hierarchical data structure and improves model estimates.

All possible risk factors were included in the initial stages. This included the risk of tick bites, the risk of infection from collecting and eating an engorged tick, contact with wild and domestic animals, contact with blood or animal tissues, as well as contact with a sick person.

Colinearity was examined in the glmm without random effects by calculating the adjusted generalised variance inflation factor (adjusted gVIF) using the car library (Fox John & Weisberg Sanford, 2019). This measures how much the variance of the regression coefficient is inflated due to the collinearity of variables. For values below 1.5, there is only low collinearity, and these variables can stay in the analysis.

A forward stepwise selection was used, where all possible risk variables were added sequentially, to see if the model improved with increasing complexity. To make the model more stable, the optimiser bobyqa was added to the function.

I started with a glmm including districts, sex and age, as the basic variables to control for differences. Subsequent variables were added individually, and the resulting model was compared to the previous one using ANOVA. The ANOVA test compares log likelihoods to determine how well the model fits the real data. Additionally, the Akaike Information Criterion (AIC) was used to measure fit and complexity by incorporating the number of variables and the maximum likelihood of the model. A lower value represents a better model. A detailed stepwise explanation of the multivariable analysis structure is visually presented in Figure 5.4.

The final model is presented as a graph using the odds ratios (ORs) and the confidence intervals, which were calculated using the Wald test.

A simple catalytic force of infection (FOI) model was fitted with the age-specific seropositivity data to observe how CCHFV exposure changes with age (Function 5.6). This model describes the rate at which individuals seroconvert (Hens *et al.* 2010), but does not include antibody waning, assumes constant FOI across age, and does not account for infection-related mortality. $P(a)$ represents the probability of seropositivity for an individual of age a, and λ denotes the FOI. The model was fitted using nonlinear least squares via the `nls()` function from base R (R Core Team, 2021), which estimates λ by minimising the residual sum of squares between the observed seropositivity outcomes and the expected values predicted by the model.

$$P(a) = 1 - e^{-\lambda a} \tag{5.6}$$

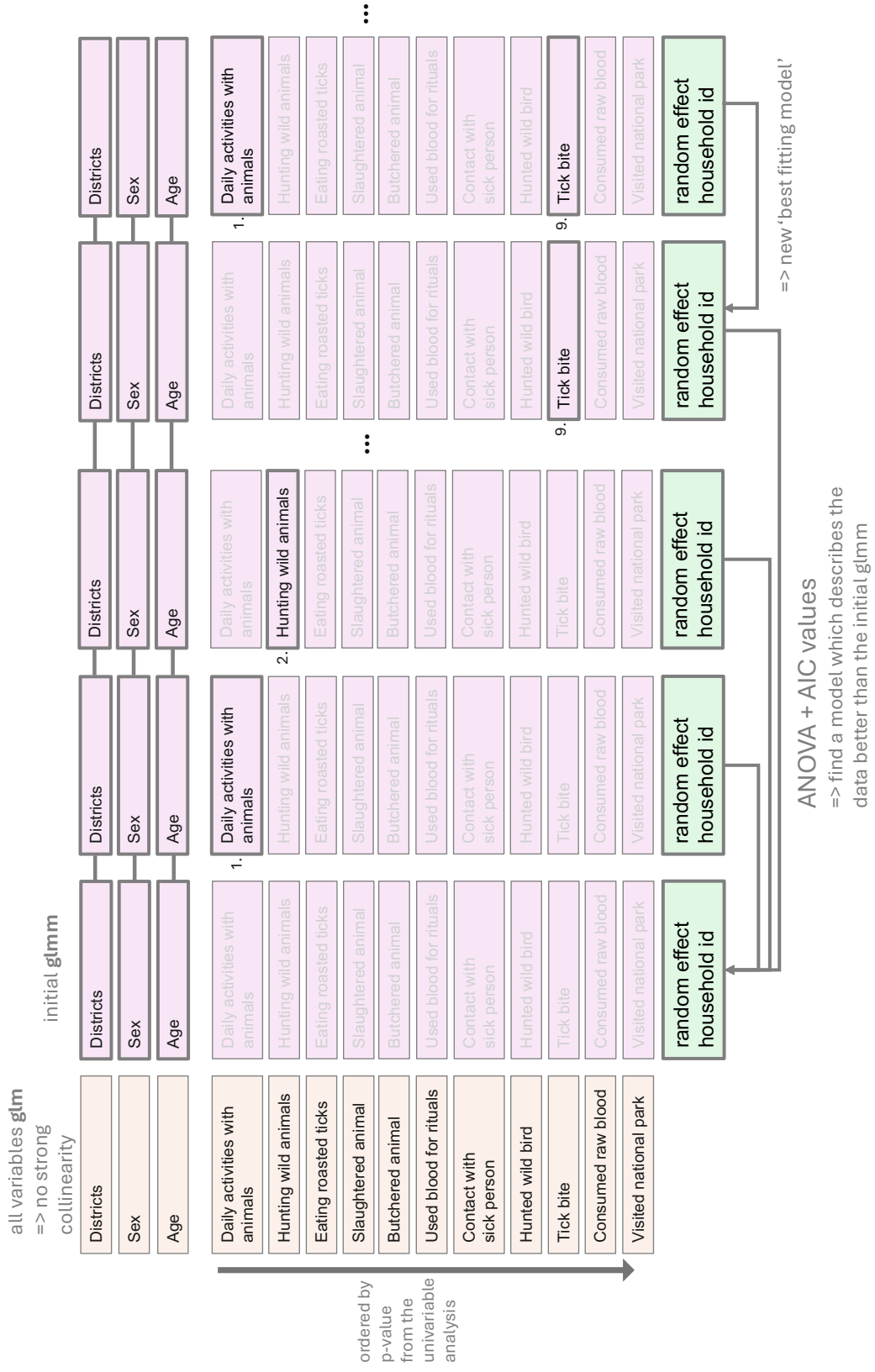

Figure 5.4: **Multivariable analysis structure.** See legend on the previous page for details.

Legend for Figure 5.4

Multivariable analysis structure. Each column presents a multivariable analysis, either a generalised linear model (glm; orange) or a generalised linear mixed model (glmm; pink; includes random effect). The analyses were conducted sequentially, including different variables, highlighted by black font and a thick surrounding. To compare the models, ANOVA and AIC values were used.

5.5 Results

Characteristics of the study population

A total of 1,059 participants from four districts of Uganda (Soroti, Kaabong, Kalangala and Kasese) were recruited by December 2024. 320 participants were intended to be recruited per district, and we reached this number in Soroti, Kaabong and Kalangala. Kasese district could not be fully recruited due to insecurity in the area, but a total of 96 participants from three villages were recruited before the study team had to stop activities, with just two of the villages having the full 10 households surveyed. Table 5.3 presents all demographic variables by district. There were more females (609/1,059; 57.5%) than males (450/1,059; 42.5%). The ages were spread evenly between the four predesigned age groups: 2 to 14 years (265/1,059; 25.0%), 15 to 27 years (262/1,059; 24.7%), 28 to 40 years (269/1,059; 25.4%) and 41 years and above (263/1,059; 24.8%). The predominant tribe varied by district. In Soroti, the Iteso tribe made up 75.2% of all participants (243/323) with multiple other tribes present in smaller numbers. In Kalangala, the Buganda were the most dominant tribe (268/320; 83.8%) and in Kasese, the Bakonzo (81/96; 84.4%) were dominant. Kaabong was the only district with all participants identifying with one tribe, the Karamojong (320/320; 100%).

44.9% (469/1,059) of our study population were in the 1st quintile in the EquityTool, which represents the poorest 20% of Uganda. 93.8% (300/320) of participants in Kaabong were in the 1st quantile, while 43.0% (133/320) of participants in Kalangala were in the highest quantile 5, representing the wealthiest quintile in Uganda.

The predominant religion was Christianity (1,019/1,059; 96.2%). Many participants had no formal school education (231/1,059; 21.8%) or only reached primary school level as their highest level of education (378/1,059; 35.7%). The main occupation of all study participants was farming, which was split between crops only (295/1,059; 27.9%) and livestock or mixed farming (174/1,059; 16.4%).

Ten households per district were recruited as 'fully recruited households', meaning that all available age groups were selected and recruited. Figure 5.5 presents only these ten fully recruited households per district, as these were used to estimate seroprevalence by district, village and household. The age structure of Uganda, as described in the methods section, was evident, with more children being present in the households than older individuals. Therefore, as part of the total population of Uganda, many more children than older individuals were not recruited, represented in the grey bars in Figure 5.5e and f.

CCHFV ELISA

Relative Optical Density values (ODs) (S/P% values) varied between 0 and 200% (Figure 5.6). An overall seropositivity of 5.3% (56/1,059) was observed in the study cohort. This ranged from 2.5% (8/320) in Kaabong, to 15.6% (15/96) in Kasese (Figure 5.5).

	Soroti (N=323)	Kaabong (N=320)	Kalangala (N=320)	Kasese (N=96)	Total (N=1059)
Sex					
male	138 (42.7%)	108 (33.8%)	163 (50.9%)	41 (42.7%)	450 (42.5%)
female	185 (57.3%)	212 (66.3%)	157 (49.1%)	55 (57.3%)	609 (57.5%)
Age category (years)					
2 - 14	81 (25.1%)	80 (25.0%)	80 (25.0%)	24 (25.0%)	265 (25.0%)
15 - 27	81 (25.1%)	80 (25.0%)	77 (24.1%)	24 (25.0%)	262 (24.7%)
28 - 40	81 (25.1%)	80 (25.0%)	84 (26.3%)	24 (25.0%)	269 (25.4%)
41 and above	80 (24.8%)	80 (25.0%)	79 (24.7%)	24 (25.0%)	263 (24.8%)
Tribe					
Bakonzo	0 (0%)	0 (0%)	5 (1.6%)	81 (84.4%)	86 (8.1%)
Banyankole	0 (0%)	0 (0%)	23 (7.2%)	4 (4.2%)	27 (2.5%)
Buganda	4 (1.2%)	0 (0%)	268 (83.8%)	0 (0%)	272 (25.7%)
Iteso	243 (75.2%)	0 (0%)	3 (0.9%)	0 (0%)	246 (23.2%)
Karamojong	1 (0.3%)	320 (100%)	0 (0%)	0 (0%)	321 (30.3%)
Kumam	70 (21.7%)	0 (0%)	0 (0%)	0 (0%)	70 (6.6%)
Other	5 (1.5%)	0 (0%)	21 (6.6%)	11 (11.5%)	37 (3.5%)
National Quintile					
Quantile 1 (poorest 20%)	133 (41.2%)	300 (93.8%)	19 (6.1%)	17 (18.5%)	469 (44.9%)
Quantile 2	49 (15.2%)	12 (3.8%)	39 (12.6%)	24 (26.1%)	124 (11.9%)
Quantile 3	50 (15.5%)	8 (2.5%)	60 (19.4%)	21 (22.8%)	139 (13.3%)
Quantile 4	62 (19.2%)	0 (0%)	58 (18.8%)	8 (8.7%)	128 (12.3%)
Quantile 5	29 (9.0%)	0 (0%)	133 (43.0%)	22 (23.9%)	184 (17.6%)
Not recorded	0 (0%)	0 (0%)	11 (3.4%)	4 (4.2%)	15 (1.4%)
Religion					
Christianity	318 (98.5%)	313 (97.8%)	293 (91.6%)	95 (99.0%)	1019 (96.2%)
Islam	3 (0.9%)	4 (1.3%)	25 (7.8%)	0 (0%)	32 (3.0%)
Traditional	0 (0%)	2 (0.6%)	1 (0.3%)	0 (0%)	3 (0.3%)
None	2 (0.6%)	1 (0.3%)	1 (0.3%)	1 (1.0%)	5 (0.5%)
Higest educational level					
Below age 12	73 (22.6%)	71 (22.2%)	73 (22.8%)	19 (19.8%)	236 (22.3%)
No formal education	30 (9.3%)	138 (43.1%)	39 (12.2%)	24 (25.0%)	231 (21.8%)
Primary school level	142 (44.0%)	85 (26.6%)	123 (38.4%)	28 (29.2%)	378 (35.7%)
Senior school level	56 (17.3%)	23 (7.2%)	71 (22.2%)	22 (22.9%)	172 (16.2%)
Certificate/Diploma	20 (6.2%)	2 (0.6%)	8 (2.5%)	2 (2.1%)	32 (3.0%)
University degree	2 (0.6%)	1 (0.3%)	6 (1.9%)	1 (1.0%)	10 (0.9%)
Occupation (within past year)					
Farm worker (crops only)	70 (21.7%)	173 (54.1%)	40 (12.5%)	12 (12.5%)	295 (27.9%)
Farm worker (livestock only or mixed)	105 (32.5%)	23 (7.2%)	29 (9.1%)	17 (17.7%)	174 (16.4%)
Fishing	1 (0.3%)	0 (0%)	50 (15.6%)	0 (0%)	51 (4.8%)
Student	64 (19.8%)	20 (6.3%)	55 (17.2%)	30 (31.3%)	169 (16.0%)
Other	41 (12.7%)	31 (9.7%)	81 (25.3%)	11 (11.5%)	164 (15.5%)
Below age 6	36 (11.1%)	35 (10.9%)	47 (14.7%)	3 (3.1%)	121 (11.4%)
Not employed	6 (1.9%)	38 (11.9%)	18 (5.6%)	23 (24.0%)	85 (8.0%)

Table 5.3: **Study participant demographics.** Presented by district and as total.

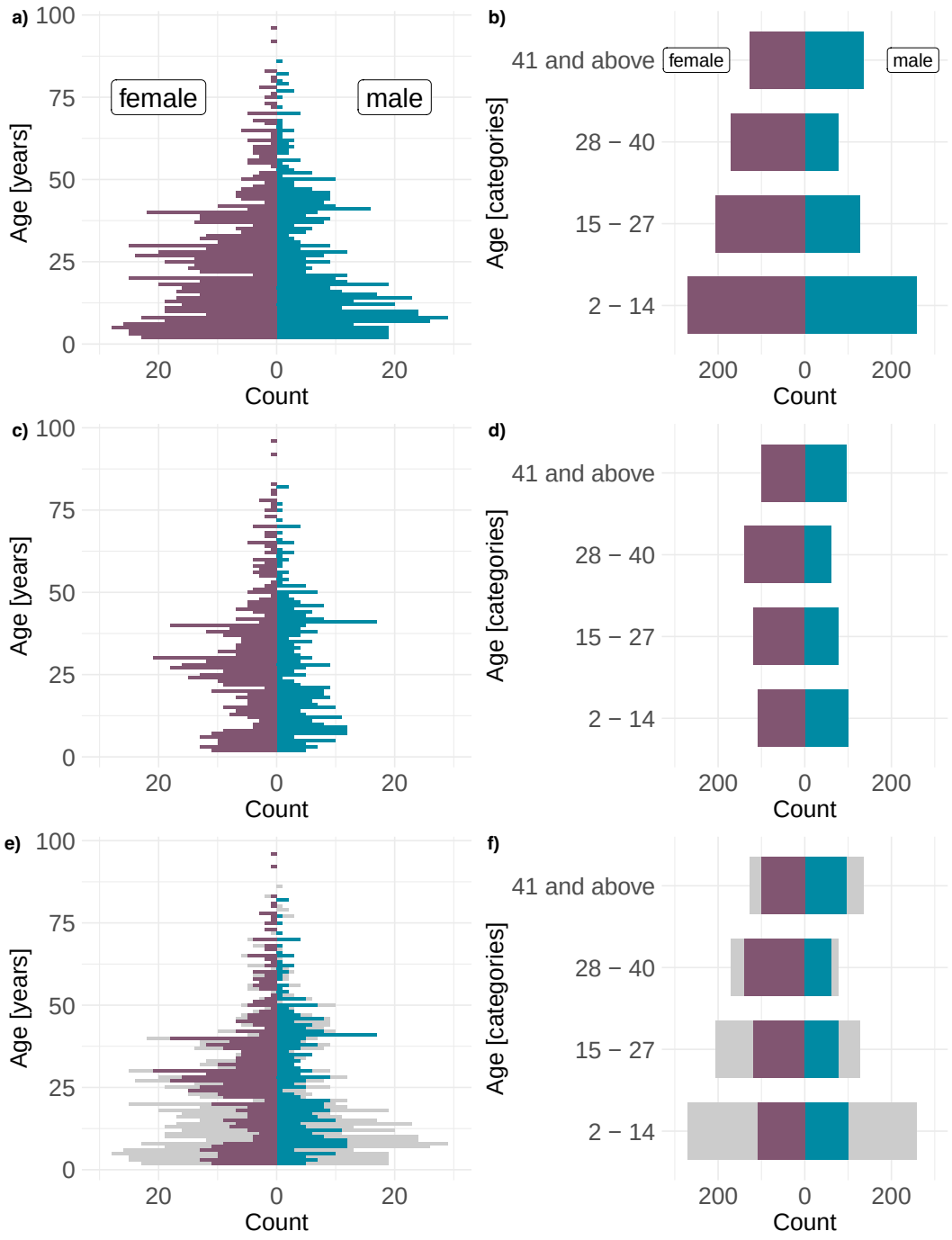

Figure 5.5: **Age structure of population and recruited participants.** Females are represented in purple, and on the left side. Males are represented in blue, and on the right side. **(a)**, **(c)**, and **(e)** present individual ages and **(b)**, **(d)**, and **(f)** present the equivalent plot for age groups. **(a)** and **(b)** represent the total population in the fully recruited households (10 per village). **(c)** and **(d)** present the recruited participants from the fully recruited households, and **(e)** and **(f)** are the plots combined, with the grey representing the total population.

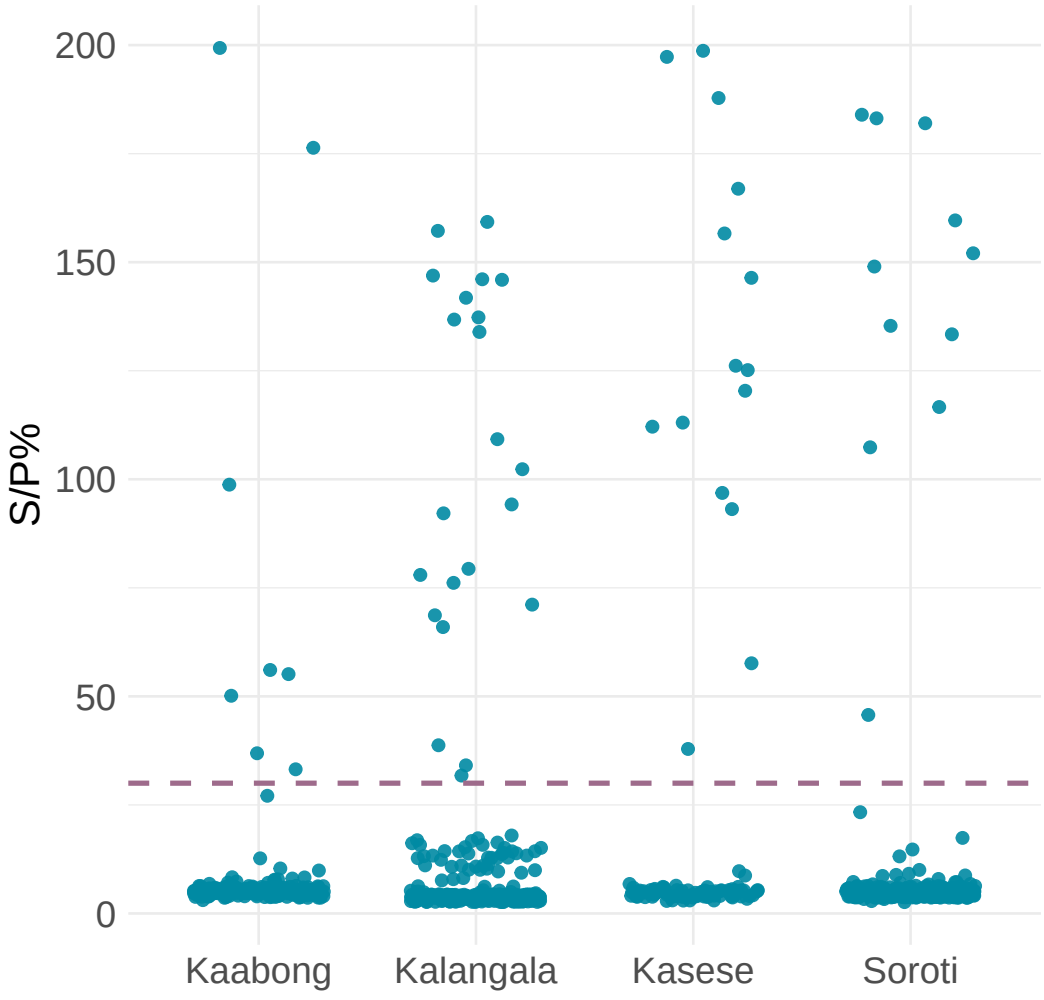

Figure 5.6: **Relative OD values (S/P% values) for CCHFV ELISA by district.** Each dot represents one participant sample, the purple line represents the cut-off of 30.

CCHFV estimated seroprevalence

To estimate seroprevalence by district, village and household, only fully recruited households were included in the analysis. This changed the observed seroprevalence slightly (presented in Table 5.4). In the same table is the estimated seroprevalence by district recorded, with 2.2% in Kaabong, 2.8% in Soroti, 4.0% in Kalangala and 18.2% in Kasese. The estimated seroprevalence by district is visualised in a barplot in Figure 5.7. Significant differences were observed between Kasese and all three other districts ($p < 0.001$).

District	**Cohort seroprevalence**	**Estimated seroprevalence for districts**	**SE**
Soroti	4.0 %	2.8 %	0.0049
Kaabong	2.6 %	2.2 %	0.0045
Kalangala	6.0 %	4.0 %	0.0152
Kasese	19.7 %	18.2 %	0.0158

Table 5.4: **Cohort and estimated seroprevalence** for all four sampled districts, including standard error (SE) from estimated seroprevalence.

The villages' estimated seroprevalences varied between 0% and 19.6% in different villages. The median percentage was 3.4% and the mean 4.5%. Figure 5.8 displays the estimated seroprevalences by village in the four districts. The highest values were detected in Kasese and Kalangala. The largest differences in seropositivity by villages were observable in Kalangala, a district characterised by multiple islands situated within Lake Victoria.

Kaabong had the largest households and Kalangala the smallest (Figure 5.9a). Soroti was diverse, with very large and small households. The highest seroprevalence in Kasese was represented again in a different format in the household representation (Figure 5.9b), with many households containing at least

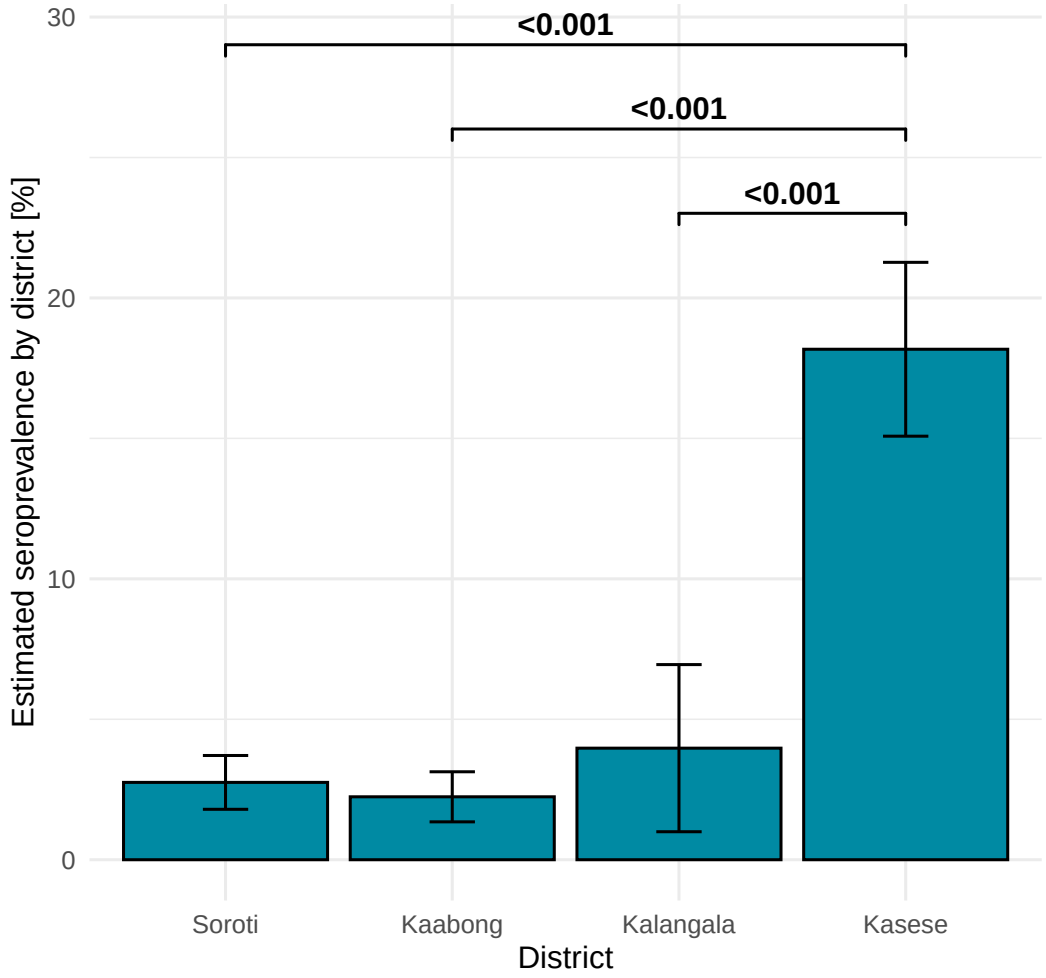

Figure 5.7: **Estimated seroprevalence for CCHFV in the four sampled districts.** The study design includes stratification by district, clustering by village and household and weights were calculated using the probability of selection. The survey library (Lumley, 2024) was used for seroprevalence estimation and the significance test (glm including complex design)

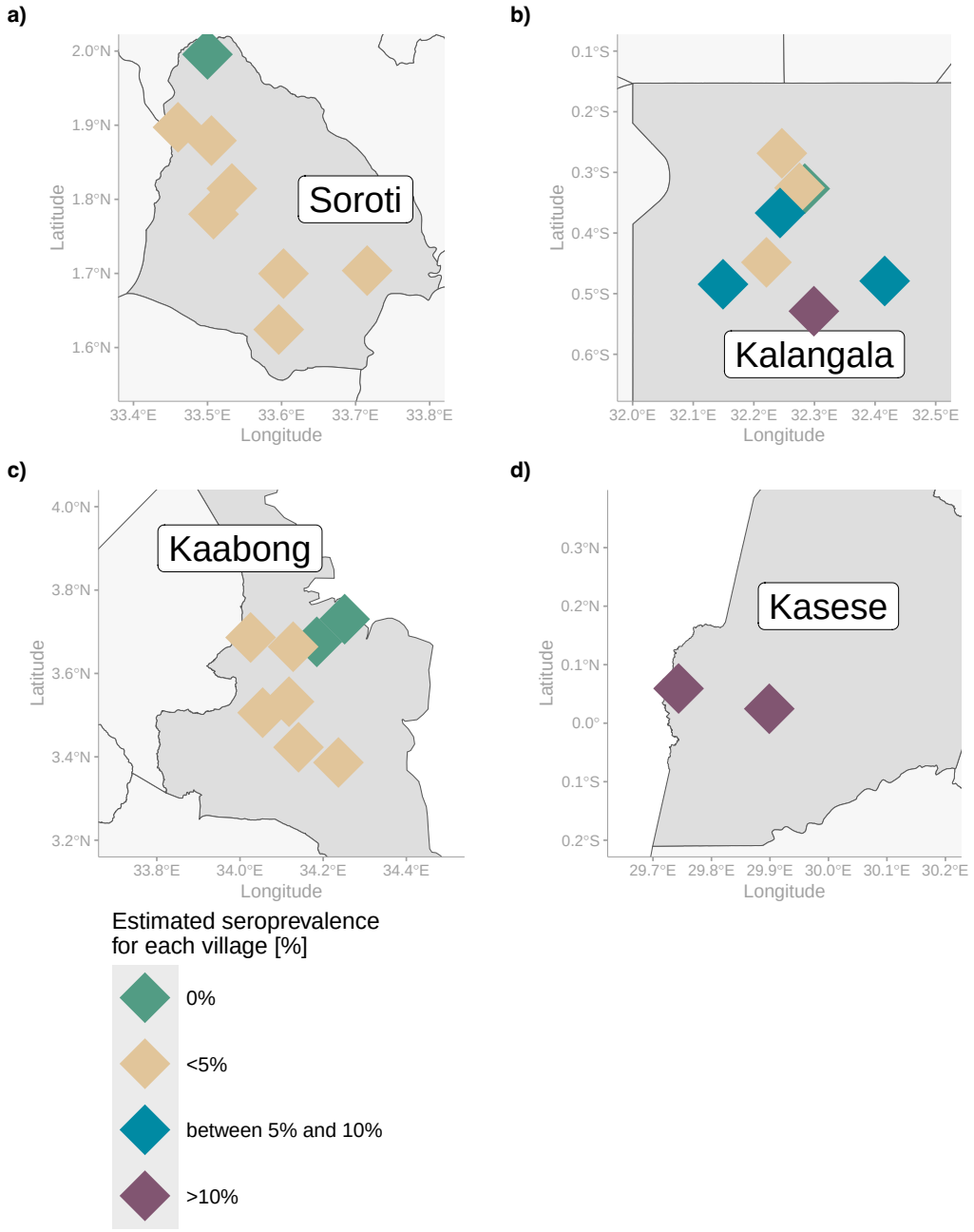

Figure 5.8: **Estimated seroprevalence for CCHFV in all villages.** The study design includes stratification by district, clustering by household and weights were calculated using the probability of selection. **(a)** Soroti, **(b)** Kalangala, **(c)** Kaabong, **(d)** Kasese.

one seropositive tested individual. The estimated seroprevalence by household is visualised in Figure 5.9c, however, with the caveat that smaller households easily reached high estimated seroprevalences when few individuals tested positive.

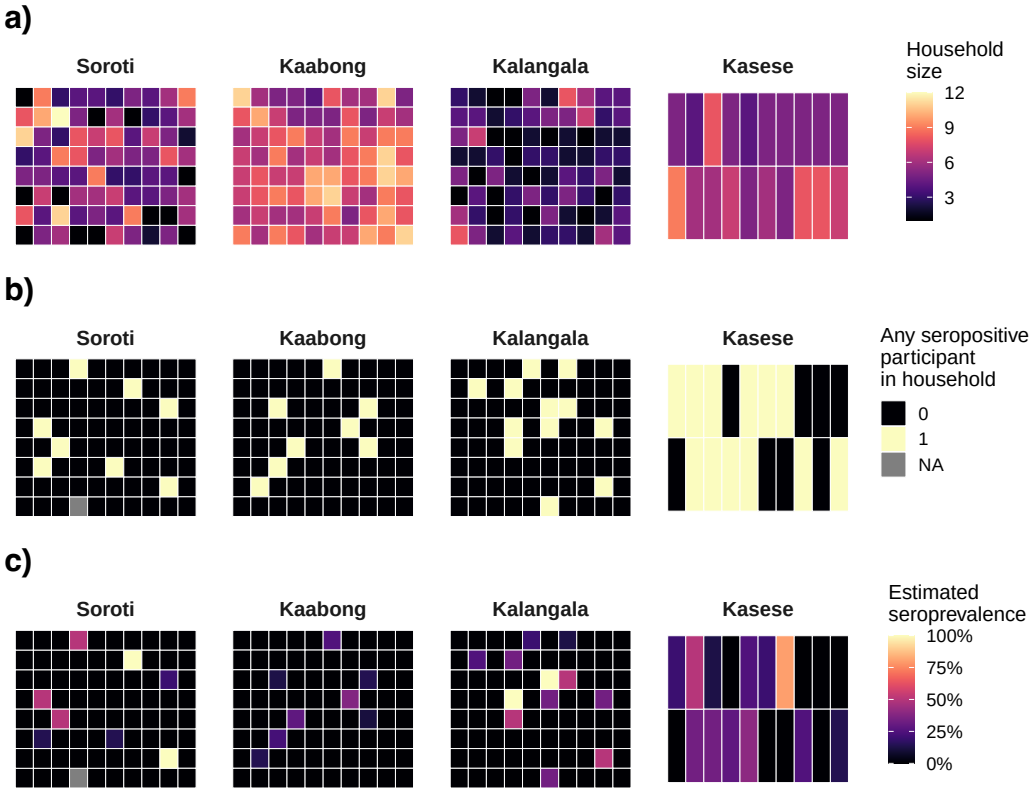

Figure 5.9: **Estimated seroprevalence for CCHFV in households.** Villages of each district are represented in their respective village, each row illustrating one village per district and each square a household. **(a)** Household size for each fully recruited household. **(b)** Calculation for at least one participant testing positive for specific antibodies against CCHFV. **(c)** Estimated seroprevalence using weights, for each household.

Risk factors for CCHFV seropositivity

For the risk factor analysis for CCHFV exposure, all participants were included. The seroprevalence tested in the study cohorts by district is presented in Figure 5.5, including possible risk factors as described in the introduction

and method section. In most districts, tick bites were commonly reported (60.6% in Kaabong and 45.2% in Soroti), except in Kalangala, with only 5 participants reporting exposure to tick bites (1.6%). Eating roasted ticks was mentioned in Kaabong (42/320; 13.1%) but not in other districts. Hunting wild animals or birds was more common in Kaabong and Soroti compared to Kalangala and Kasese. 24.6% of all participants reported to have slaughtered an animal or to have taken part in a slaughter. The majority in Soroti (205/323; 63.5%) and Kasese (69/96; 71.9%) looked after animals on a daily basis. Consuming raw blood was mentioned by 21.3% of all participants in Kaabong, with only one report in Kalangala and none in the other districts. Many people reported to have had contact with a sick person in the last year (301/1,059; 28.5%).

Conducting a multivariable analysis showed no strong collinearity between all variables. Only two variables, the districts and the tick bites variables, reached an adjusted gVIF of 1.5, the threshold for low collinearity, but it wasn't high enough to remove from the models. All adjusted gVIFs are presented in Table 5.6.

	Soroti (N=323)	Kaabong (N=320)	Kalangala (N=320)	Kasese (N=96)	Total (N=1059)
CCHFV IgG ELISA result					
CCHFV positive	11 (3.4%)	8 (2.5%)	22 (6.9%)	15 (15.6%)	56 (5.3%)
CCHFV negative	312 (96.6%)	312 (97.5%)	298 (93.1%)	81 (84.4%)	1003 (94.7%)
Tick bite ever					
Yes	146 (45.2%)	194 (60.6%)	5 (1.6%)	23 (24.0%)	368 (34.7%)
No	177 (54.8%)	126 (39.4%)	315 (98.4%)	73 (76.0%)	691 (65.3%)
Eaten roasted tick (ever)					
Yes	1 (0.3%)	42 (13.1%)	0 (0%)	0 (0%)	43 (4.1%)
No	322 (99.7%)	278 (86.9%)	320 (100%)	96 (100%)	1016 (95.9%)
Visited national park or protected area (past year)					
Yes	3 (0.9%)	20 (6.3%)	18 (5.6%)	0 (0%)	41 (3.9%)
No	320 (99.1%)	300 (93.8%)	302 (94.4%)	96 (100%)	1018 (96.1%)
Hunted wild animal (past year)					
Yes	56 (17.3%)	131 (40.9%)	2 (0.6%)	3 (3.1%)	192 (18.1%)
No	267 (82.7%)	189 (59.1%)	318 (99.4%)	93 (96.9%)	867 (81.9%)
Hunted wild bird (past year)					
Yes	70 (21.7%)	42 (13.1%)	1 (0.3%)	NA	113 (11.7%)
No	253 (78.3%)	278 (86.9%)	319 (99.7%)	NA	850 (88.3%)
Not recorded	0 (0%)	0 (0%)	0 (0%)	96 (100%)	96 (9.1%)
Slaugthered animal or took part (past year)					
Yes	186 (57.6%)	51 (15.9%)	21 (6.6%)	2 (2.1%)	260 (24.6%)
No	137 (42.4%)	269 (84.1%)	299 (93.4%)	94 (97.9%)	799 (75.4%)
Butchered animal (past year)					
Yes	82 (25.4%)	13 (4.1%)	106 (33.1%)	0 (0%)	201 (19.0%)
No	241 (74.6%)	307 (95.9%)	214 (66.9%)	96 (100%)	858 (81.0%)
Daily caring for animals					
Yes	205 (63.5%)	60 (18.8%)	108 (33.8%)	69 (71.9%)	442 (41.7%)
No	118 (36.5%)	260 (81.3%)	212 (66.3%)	27 (28.1%)	617 (58.3%)
Consumed raw blood (past year)					
Yes	0 (0%)	68 (21.3%)	1 (0.3%)	0 (0%)	69 (6.5%)
No	323 (100%)	252 (78.8%)	319 (99.7%)	96 (100%)	990 (93.5%)
Used blood or animal products in ritual (past year)					
Yes	2 (0.6%)	12 (3.8%)	0 (0%)	0 (0%)	14 (1.3%)
No	321 (99.4%)	308 (96.3%)	320 (100%)	96 (100%)	1045 (98.7%)
Contact with sick person (past year)					
Yes	107 (33.1%)	170 (53.3%)	16 (5.0%)	8 (8.3%)	301 (28.5%)
No	216 (66.9%)	149 (46.7%)	304 (95.0%)	88 (91.7%)	757 (71.6%)
Not recorded	0 (0%)	1 (0.3%)	0 (0%)	0 (0%)	1 (0.1%)

Table 5.5: **Cohort seroprevalence for CCHFV and risk factors by districts.**

Variable	**Adjusted gVIF**
District	1.55
Sex	1.13
Age category	1.03
Tick bite	1.52
Eaten roasted tick	1.18
Visited national park or protected area	1.00
Daily caring for animals	1.12
Hunted wild animal	1.28
Hunted wild bird	1.15
Butchered animal	1.22
Consumed raw blood	1.42
Used blood or animal products in ritual	1.15
Contact with sick person	1.25

Table 5.6: **Adjusted generalised variance inflation factor (gVIF) for all variables.**

Table 5.7 and Table 5.8 show all variables analysed in a univariable regression, against the outcome of CCHFV seropositivity. In this analysis, district and age were significant. The highest odds for seropositivity were in Kasese district (AOR=6.36; 95% CI=2.14-18.9; $p < 0.001$), and the oldest age group (41 and above) presented with the highest odds within varying age groups (AOR=32.6; 95% CI=7.84-136; $p < 0.001$). A multivariable analysis was carried out, correcting for district, sex and age. Variables with the lowest p-values were added to the analysis sequentially. Addition of the tick bite variable improved the model, and was carried forward to the final analysis (Table 5.7 and visual in Figure 5.10). As in the univariable analysis, only district (Kasese; AOR=11.2; 95% CI=2.66-47.1; $p = 0.002$) and age (41 and above; AOR=9.19; 95% CI=2.99-28.3; $p < 0.001$) were significant in this interim multi-variable analysis.

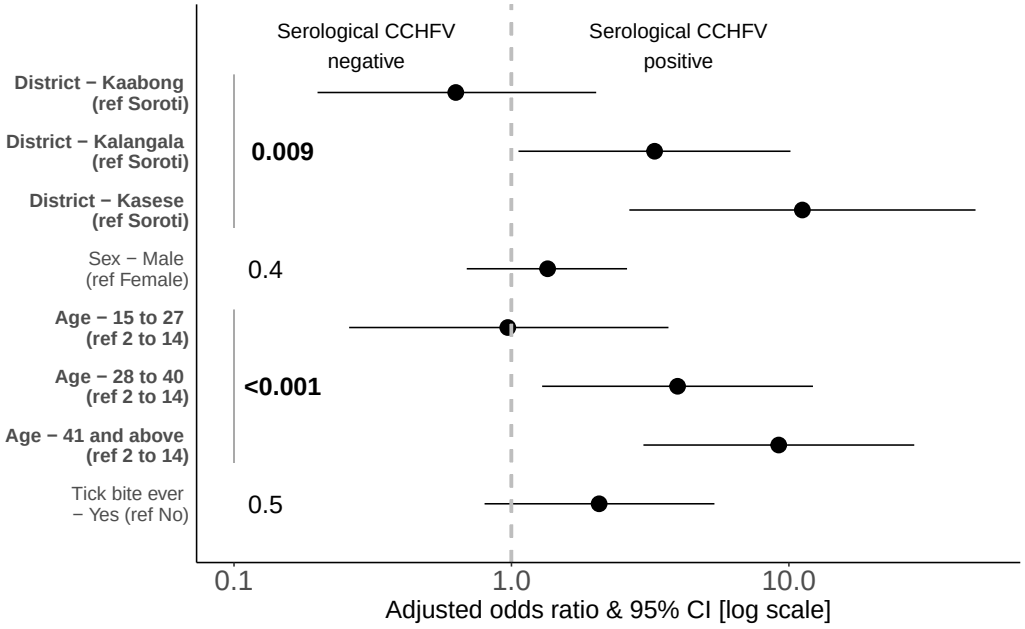

Figure 5.10: **Risk factor analysis for CCHFV.** Graph shows odds ratios with 95% confidence intervals and p-values.

Characteristic	CCHFV positive N = 56[1]	CCHFV negative N = 1,003[1]	Univariable regression			Multivariable regression		
			OR[2]	95% CI	Univariable p.value	AOR[3]	95% CI	Multivariable p.value
District					**<0.001**			**0.002**
Kaabong	8 (2.5%)	312 (97.5%)	0.72	0.26, 2.01		0.63	0.20, 2.02	
Kalangala	22 (6.9%)	298 (93.1%)	2.16	0.93, 5.04		3.28	1.06, 10.1	
Kasese	15 (15.6%)	81 (84.4%)	6.36	2.14, 18.9		11.2	2.66, 47.1	
Soroti	11 (3.4%)	312 (96.6%)	—	—		—	—	
Sex					0.15			0.4
male	30 (6.7%)	420 (93.3%)	1.55	0.86, 2.82		1.35	0.69, 2.61	
female	26 (4.3%)	583 (95.7%)	—	—		—	—	
Age category					**<0.001**			**<0.001**
2 - 14	5 (1.9%)	260 (98.1%)	—	—		—	—	
15 - 27	5 (1.9%)	257 (98.1%)	0.83	0.17, 4.07		0.97	0.26, 3.68	
28 - 40	16 (5.9%)	253 (94.1%)	7.45	1.93, 28.8		3.97	1.29, 12.2	
41 and above	30 (11.4%)	233 (88.6%)	32.6	7.84, 136		9.19	2.99, 28.3	
Tick bite ever					0.8			0.13
Yes	18 (4.9%)	350 (95.1%)	1.09	0.53, 2.22		2.07	0.80, 5.39	
No	38 (5.5%)	653 (94.5%)	—	—		—	—	

[1] n (%)
[2] OR = Odds Ratio
[3] AOR = Adjusted Odds Ratio
Abbreviations: CI = Confidence Interval, OR = Odds Ratio, NA

Table 5.7: **Multivariable analysis for risk factors for CCHFV.**

Characteristic	CCHFV positive N = 56[1]	CCHFV negative N = 1,003[1]	Univariable regression OR[2]	95% CI	Univariable p.value
Eaten roasted tick (ever)					0.5
Yes	1 (2.3%)	42 (97.7%)	0.46	0.05, 3.92	
No	55 (5.4%)	961 (94.6%)	—	—	
Visited national park or protected area					>0.9
Yes	0 (0.0%)	41 (100.0%)	0.00	0.00, Inf	
No	56 (5.5%)	962 (94.5%)	—	—	
Daily caring for animals					0.14
Yes	28 (6.3%)	414 (93.7%)	1.68	0.85, 3.33	
No	28 (4.5%)	589 (95.5%)	—	—	
Hunted wild animal					0.3
Yes	6 (3.1%)	186 (96.9%)	0.58	0.22, 1.52	
No	50 (5.8%)	817 (94.2%)	—	—	
Hunted wild bird					0.7
Yes	4 (3.5%)	109 (96.5%)	0.67	0.10, 4.43	
No	37 (4.4%)	813 (95.6%)	—	—	
Slaugthered animal or took part					0.5
Yes	10 (3.8%)	250 (96.2%)	0.77	0.34, 1.72	
No	46 (5.8%)	753 (94.2%)	—	—	
Butchered animal					0.5
Yes	12 (6.0%)	189 (94.0%)	1.34	0.62, 2.87	
No	44 (5.1%)	814 (94.9%)	—	—	
Consumed raw blood					>0.9
Yes	3 (4.3%)	66 (95.7%)	1.08	0.28, 4.21	
No	53 (5.4%)	937 (94.6%)	—	—	
Used blood or animal products in ritual					0.5
Yes	1 (7.1%)	13 (92.9%)	2.17	0.21, 22.7	
No	55 (5.3%)	990 (94.7%)	—	—	
Contact with sick person					0.5
Yes	11 (3.7%)	290 (96.3%)	0.75	0.35, 1.61	
No	45 (5.9%)	712 (94.1%)	—	—	

[1] n (%)
[2] OR = Odds Ratio
Abbreviations: CI = Confidence Interval, OR = Odds Ratio, NA

Table 5.8: **All risk variables analysed by univariable analysis for CCHFV exposure**

Force of infection

Seroprevalence plotted by age is shown in Figure 5.11a, including a catalytic model representing estimated FOI. We estimated 194 seroconversions per 100,000 susceptible individuals per year ($\lambda = 0.00194$). The model fit was statistically significant ($p < 0.001$), in keeping with age being associated with increased seropositivity rates. Figure 5.11b presents the same model and data, but the age is presented within the age groups used during recruitment.

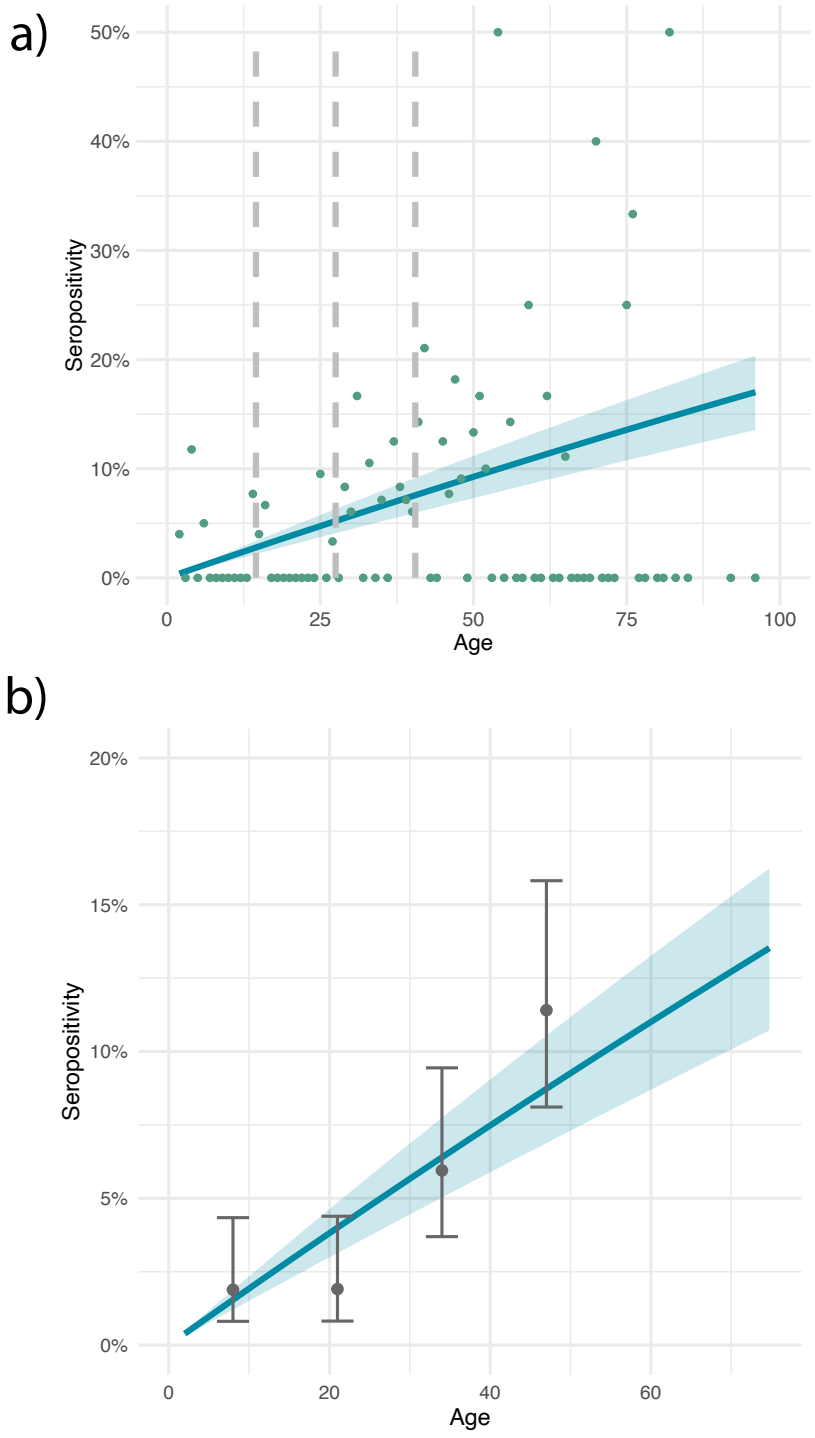

Figure 5.11: **Force of infection for CCHFV in study cohort. a)** Graph displays seroprevalence by age, catalytic model representing force of infection (FOI), including confidence intervals. The grey dotted bars present the borders of the age groups during recruitment. Each quarter contains a quarter of all participants. **b)** Seroprevalence by age group, including FOI.

5.6 Discussion

In this chapter, I describe the analysis of a cross-sectional, household-based, and randomised serosurvey for CCHFV exposure in four districts of Uganda, which were selected based on distinct environmental and socioecological variables through K-prototype analysis. Significant differences were shown in estimated exposure rates between Kasese district and the other three districts. In addition, we detected a significant increase in seroprevalence with age.

A significantly higher estimated seroprevalence for CCHFV was observed in Kasese district, compared to the other study areas. Kasese district lies in the Western Region of Uganda, bordering the Democratic Republic of the Congo (DRC) to the west, and includes large areas of two national parks within its borders (detailed description in Chapter 3). Kasese district reported the highest percentage of people who care daily for domestic animals (72%), compared with Soroti, Kalangala and Kaabong. Tick bites were prevalent, as highlighted in this survey and strongly supported by the qualitative study (Chapter 4). However, tick bites were more often reported in Soroti and Kaabong, where around half of the participants reported having ever been bitten by ticks, compared to only 24% in Kasese district. The reasons for the high seropositivity in Kasese remain unknown, but may be explained by environmental and/or socioecological conditions in the district. Lule *et al.* 2022 reported high environmental suitablitiy for *Rhipicephalus appendiculatus* and *Amblyomma variegatum* in Kasese District based on their modelling studies. These tick species have previously been reported as testing positive for CCHFV in Uganda (Atim, Ashraf, *et al.* 2023; Lule *et al.* 2022).

Several seroprevalence studies have suggested spatial variance in seropositivity in Uganda. Table 5.9 presents a summary of identified scientific papers which conducted seroprevalence and risk analysis for CCHFV in Uganda. Although all studies varied in location, study design, assays used, and other details, the

results are similar in many aspects. In preceding studies, including those with multiple study sites (at district or regional level), seroprevalence varies significantly. Kasese district hasn't been included in preceding studies for human exposure, other than our HCWs study presented in Chapter 2. In Chapter 2, a different assay was used (VectorBest), and the study design focused on comparing HCWs with community members. The seroprevalence in the community members was 15.2%, which was similar (slightly lower) compared to the estimated 18.2% in the study presented in this chapter. This small difference may highlight differences due to the use of different assays and a different study design. However, in both studies, prevalence is consistently high, and emphasises that endemic exposure to CCHFV is present in this area.

While the study was not powered to identify within-district variations, notable variation between villages was observed within Kalangala district. Kalangala is an island district, as described in Chapter 3, and interestingly presents substantial differences in seroprevalence between villages on different islands. Study participants in the focus group discussions (FGDs) mentioned that islands vary significantly in their surroundings, and the islands of Kalangala are interesting study sites to investigate socioecological and historical exposure factors in a similar environmental setting. Other districts analysed had similar estimated seroprevalence within villages, in keeping with homogenous exposure risks throughout these districts.

Estimated seroprevalence indicating CCHFV exposure within households of each of the four districts exhibited the same trends as in the village analysis, other than in Kalangala, where a larger heterogeneity between villages was evident and merits future detailed investigation.

Mihalakakos *et al.* 2025 investigated household members in a longitudinal seroprevalence study in Southern Uganda, and revealed heterogeneity in seropositivity within households. This finding is similar to that presented in this dataset and supports the observation that intrafamily transmissions are rela-

Table 5.9: Seroprevalence studies for CCHFV in Uganda

Authors	**Country**	**District/Area**	**Species**	**Study population**	**Seroprevalence**	**Assay**
Mihalakakos *et al.* 2025	Uganda	Masaka Region	Human	Fishing, agrarian and trading communities	4.25% (range within study communities between 2.25% and 7.75%)	ID Screen + in-house ELISA
Atim, Niebel, *et al.* 2023	Uganda	Lyantonde	Cattle	From farms which were a possible origin of a human outbreak	94.0%	ID Screen
			Goats		89.3%	ID Screen
Telford *et al.* 2023	Uganda	28 districts	Cattle	From herds	16.9%	?
			Goats	From herds	48.7%	?
			Sheep	From herds	49.2%	?
Lule *et al.* 2022	Uganda	Kampala	Human	Abattoir workers	10.3%	VectorBest
		Diverse districts	Cattle	At abattoirs	69.7%	ID Screen

Continued from Table 5.9

Authors	Country	District/Area	Species	Study population	Seroprevalence	Assay
Atim *et al.* 2022	Uganda	Arua & Nakaseke	Human	Farming communities + controls	27.6%	VectorBest
			Cattle	From households	91.8%	ID Screen
			Goats	From households	75.2%	ID Screen
			Dogs	From households	56.2%	ID Screen
Balinandi *et al.* 2021	Uganda	Kasese, Hoima, Gulu, Soroti, Moroto	Cattle	Random sampling frame	75.0% & 12.6%	ID Screen & in-house ELISA
Rodhain *et al.* 1989	Uganda	Karamoja (Nakapiripirit district)	Human	From villages	2.2%	Immunofluorescent assay

tively rare.

We detected a significant increase in seroprevalence with age, presented in the univariable and multivariable regression analyses, as well as through modelling the FOI. CCHFV exposure has been shown to increase with age, in two other studies in Uganda (Atim *et al.* 2022; Mihalakakos *et al.* 2025). Importantly, these results support the assumption of cumulative exposure with age, consistent with endemic transmission patterns of CCHFV. Our study results are supported by evidence of reported CCHFV cases during an outbreak of Sudan virus (SUDV) in the country, which otherwise might have gone undetected (Balinandi *et al.* 2024). Balinandi *et al.* 2024 mentioned the unusually high number of CCHFV case reports, highlighting endemic transmission and suggesting incomplete detection of all cases. A long period of endemicity is suggested by historical cases of CCHFV, reported throughout the country (Simpson *et al.* 1967). From a public health perspective, particularly regarding disease prevention and tick control, it is important to emphasise that exposure occurs regularly and not only during declared outbreaks and that cases of disease are likely to be significantly under-reported.

While the FOI model assumes a constant force of infection, exploratory data (Figure 5.6b) suggest that exposure rate may vary somewhat across age groups, highlighting age groups most prone to tick bites or exposure to CCHFV through direct contact. A larger sample size per district or the recruitment of additional districts would increase the number of positive participants and enable such an analysis. A more flexible modelling approach, such as the one used by de Glanville *et al.* (2022), could also be adapted in future work to account for age-related variation as well as spatial variation in our study population.

The transmission of CCHFV from ticks to animals and humans has been previously well-described in the literature (Hoogstraal, 1979; Lule *et al.* 2022). In this interim analysis of a larger study, we show that tick bites may partly explain differences in seropositivity in our study population with an adjusted

odds ratio of 2.07 [95%CI: 0.80 - 5.39]; however, with a non-significant p-value (as might be anticipated in an interim analysis). This finding may also reflect under-reporting of tick bites in this population and a lack of awareness of risk. To improve tick bite reporting, a thorough educational programme should be conducted, prior to recruitment for public health awareness and for future studies, with the intention of raising awareness and ensuring more precise reporting.

In this study, we initially planned to use the VectorBest assay (based on whole virus) for diagnosis. However, we were required to switch to ID Screen® CCHF Double Antigen Multi-species ELISA (IDvet, Grabels, France) due to difficulties in the procurement of the original assay. We subsequently assessed the ID Screen® assay with a validation experiment including parallel assessment of ten convalescent Ugandan serum samples and available cohort samples (Leah Owen - personal communication). The assay has been previously found to be highly specific (reported specificity by Karaaslan *et al.* 2025 of 99.7%) and the sensitivity has been reported as 95.2% in hospitalised patients (Karaaslan *et al.* 2025). We detected a sensitivity of 100% in our ten convalescent samples from Ugandan hospitalised patients.

However, given the lower seroprevalence found compared to Vector Best, it is possible that the assay lacks sensitivity in milder or asymptomatic infections. Antibodies with lower avidity in recent infections or without a strong immune response could also be missed, as well as some people who might not produce antibodies against the nucleoprotein of CCHFV, which is used as the antigen in the ID Screen® (Maze *et al.* 2025). Further research should be conducted in this area, and further evaluation of diagnostic assays is indicated and planned in future work.

In order to minimise difficulties during recruitment and the recording of risk factors, we trained local study teams in each district. This ensured the necessary local knowledge, including orientation within the district and cultural

norms, within the team and importantly that the team spoke and understood the local language of the participants and could undertake informed consent and conduct the questionnaire accurately, as well as being able to answer any questions the participants may have had at consent and throughout the survey. However, with different teams and different languages, some differences in the interpretation of the questionnaire could have resulted in some bias in the generated datasets. We aimed to maximise consistency with extensive training and follow-up, conducted both in person and online.

Trust is a critical aspect of conducting research in communities (Sapienza *et al.* 2007), which we established with the help of the local study teams. However, it is possible that age or sex differences may have inhibited participants from opening up about sensitive matters. Illegal behaviour such as poaching, or questions with associated stigma like eating raw meat or engorged ticks are likely to have been under-estimated.

Finally, as the questions asked about behaviours within the past year of the recruitment, participants might also forget, over- or underestimate their occurrences, as they are only recollected from memory.

Our study has several further potential limitations. First, an increased sample size by district and the recruitment of more districts will improve the power to identify significant differences in seroprevalence by district or region. We anticipate stronger results in our future multivariable analysis and will model more complex variables once the study is complete.

Another consideration is that seroprevalence has to be interpreted with care, due to the potential for cross-reactivity with other related viruses (Atim *et al.* 2022). However, the nairoviruses that we have previously identified in ticks (Nairobi sheep disease virus (NSDV) and Dugbe virus) are largely confined to domestic animals. Additionally, some participants may not seroconvert or exhibit antibody decay (Mihalakakos *et al.* 2025; Shepherd *et al.* 1989).

Future incidence studies identifying CCHFV RNA in participants, incorporating health-seeking behaviour, would increase the knowledge gap on CCHFV infections, are planned and could answer questions around exposure risks.

In the wider study, we carried out both human recruitment and parallel sampling of domestic animals. In addition, ticks were collected both from animals and the environment. This rich dataset will be combined in risk analyses to estimate FOI and seroprevalence in future work. We hope to investigate correlations between human and animal seroprevalence, as well as correlations to CCHFV presence in ticks or *Nairovirus* diversity in ticks.

5.7 Conclusions

This chapter has set a good groundwork, including a statistical framework for further analyses on the full dataset, which will be generated at the end of recruitment for this study. In conclusion, we confirmed an exceptionally high exposure risk in Kasese district for CCHFV and the endemic circulation of CCHFV in other study districts.

Chapter 6

Development of a public engagement tool for vector-borne diseases in Uganda

6.1 Acknowledgements

This work was conducted in close collaboration with the Games and Gaming Lab of the University of Glasgow and other researchers and support staff at the University of Glasgow. A complete list of co-authors and contributions of the published report is listed in Table 6.1.

This study was funded by my personal grant as part of the Wellcome Trust PhD funding (218518/Z/19/Z), and additional funding was received from the ArtsLab via UofGGamesLab and the Wellcome Centre for Integrative Parasitology Public Engagement Fund.

	Contributions
Marina Kugler	Creating, planning and leading the project
Peter Watson	Designing board
Rachel Porteous, Joanne Power, Raheema Chunara, Hannah Bialic, Lois Mason, Lazaaro Mujumbusi, Stella A Atim, Poppy Lamberton, Emma C Thomson, Timothy Peacock	Helping and guiding through the project
Ben Ssebiranda	Producing boardgames in Uganda
Richard Muhumuza, Titus Apangu, Edward Obicho, Peter L Lotyang, Joshua Muhindo	Translating boardgame
Alice Cowley	Creating CVR webpage for Vector Ludo

Table 6.1: Contributions to Vector Ludo.

6.2 Introduction

Research communication is an integral part of any research project. Educating communities about the project and its background is an essential part of obtaining informed consent for participation. Alongside the immediate goals of conducting a study, there should also be a broader aim of bringing community members closer to science and the work of scientists. This chapter aimed to develop a tool designed to bridge and facilitate these conversations engagingly and playfully.

6.3 Vector Ludo board game

This project began following initial conversations about how the arts and science departments at the University of Glasgow could collaborate on projects. Quite quickly, we got together a multidisciplinary team of researchers, designers and game developers and worked on the project alongside our individual main aims.

We decided to create a game inspired by the very popular board game Ludo, widely played by both old and young people in Uganda, to incorporate educational facets. The goal of the game is to bring your tokens safely home while navigating four areas that feature different disease-transmitting vectors that are common in Uganda and other countries in Sub-Saharan Africa. Special fields on the board delay or stop players when they encounter a vector, or offer advantages when players follow disease prevention practices.

We chose the original colour palette (green, yellow, red, and blue) and matched the vectors based on their environment: black flies transmitting *Onchocerca volvulus* (causing river blindness), ticks transmitting Crimean-Congo haemorrhagic fever virus (CCHFV) as discussed in this PhD, mosquitoes transmitting

Plasmodium spp. (causing malaria), and snails as hosts for *Schistosoma mansoni* (causing schistosomiasis). Facts and educational messages were discussed within our team and the wider research communities at the Uganda Virus Research Institute (UVRI) and the institutes of the participating researchers.

The game is available in English and six local languages commonly spoken in our study districts in Uganda: Luganda, Ateso, Karamojong, Lugbara, Lusoga, and Lukhonzho. The translations were facilitated by team members from the other studies presented in this PhD, with expertise in both science and science communication. This multilingual approach makes the game accessible to a wide range of communities. The English version of the board is presented in Figure 6.1.

The full report, including the rules, printable tokens, and all translated boards, is available to download from the University of Glasgow's open platform Enlighten (https://eprints.gla.ac.uk/343682/). Additionally, the project and the process are presented on the webpage on the MRC-University of Glasgow Centre for Virus Research (CVR) public engagement section (https://cvr-engagement.co.uk/vector-ludo).

6.4 Distribution

During the recruitment period for the serosurvey described in Chapter 5 in Soroti and Kaabong, we commissioned custom-made games featuring wooden boards with a glass cover, available in English, Ateso and Karamojong. These were used to play and engage with communities. The design was practical and portable, allowing the game to be played on any surface. We distributed the boards to villages, where they gathered groups of children and teenagers who were eager to learn and play. Each session included a discussion about the diseases and vectors featured on the board, followed by a game of Vector Ludo.

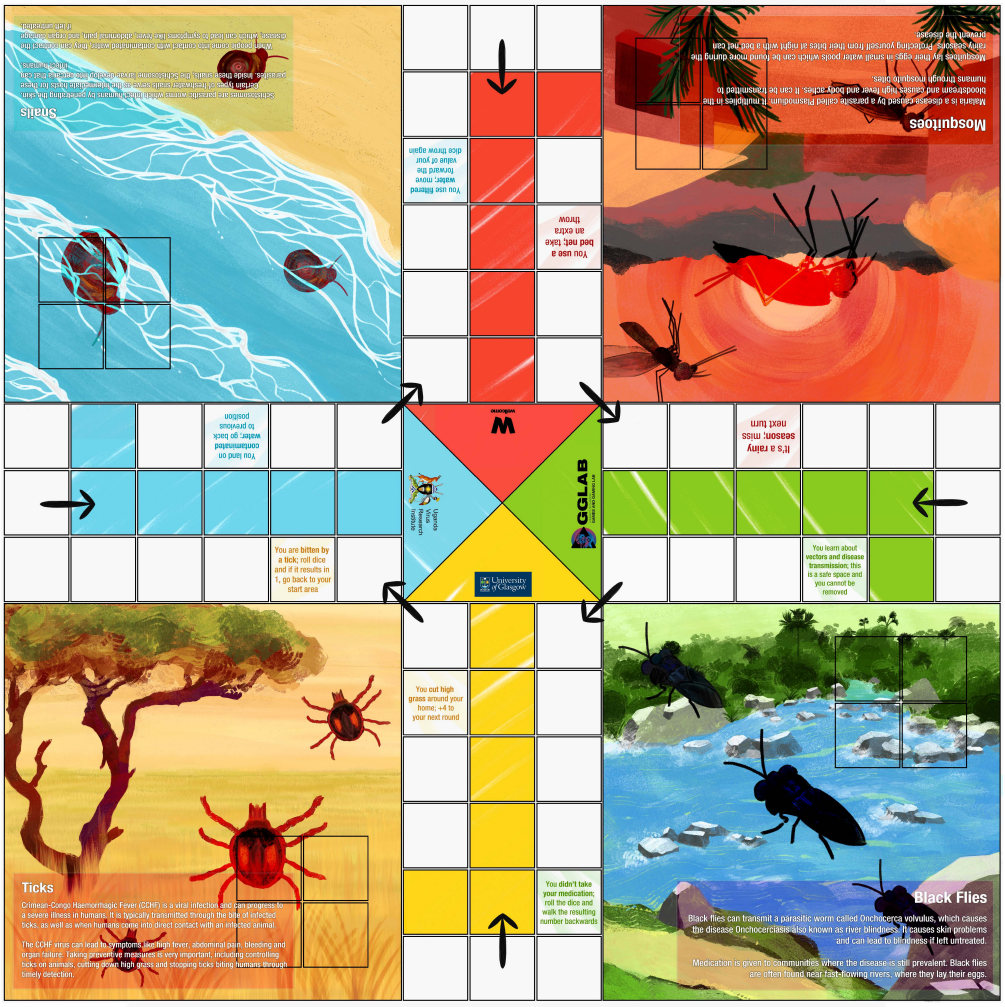

Figure 6.1: **Vector Ludo boardgame.**

Example sessions are shown in Figure 6.2, held in Kachumbala and Kaabong.

We also participated in the Explorathon in 2024, a public engagement event hosted by the University of Glasgow, which featured various projects from across the University.

6.5 Discussion

Vector Ludo quickly became a valuable tool for education, community engagement, and for sparking meaningful conversations about vector-borne diseases and their prevention in Uganda. We hope this collaborative effort will lead to many more interactions and gameplay sessions, helping to translate research into accessible and engaging communication with communities.

A structured evaluation study should be conducted in Ugandan communities to assess the impact of the Vector Ludo and refine engagement strategies. Nowbuth *et al.* 2023 highlighted the importance of measuring the effectiveness of such interventions. We encourage researchers in Uganda and other Sub-Saharan African countries to download, use and evaluate the tool.

6.6 Other efforts in public engagement

During this PhD, I participated in and contributed to scientific conferences in the UK and internationally, and also entered the Three Minute Thesis (3MT) Competition. This event showcases the projects of PhD students globally. In three minutes, I presented my project to an academic audience from diverse backgrounds. The slide I used for my talk is presented in Figure 6.3, illustrating a simplified transmission cycle for CCHFV.

Additionally, I communicated my research and my PhD experience to a non-

a)

b)

Figure 6.2: **Playing season with Vector Ludo.** A) A visit to Amuno, a non-profit organisation in Kachumbala. B) A game with the trainee nurses from Kaabong hospital during a break from recruitment in Kaabong district.

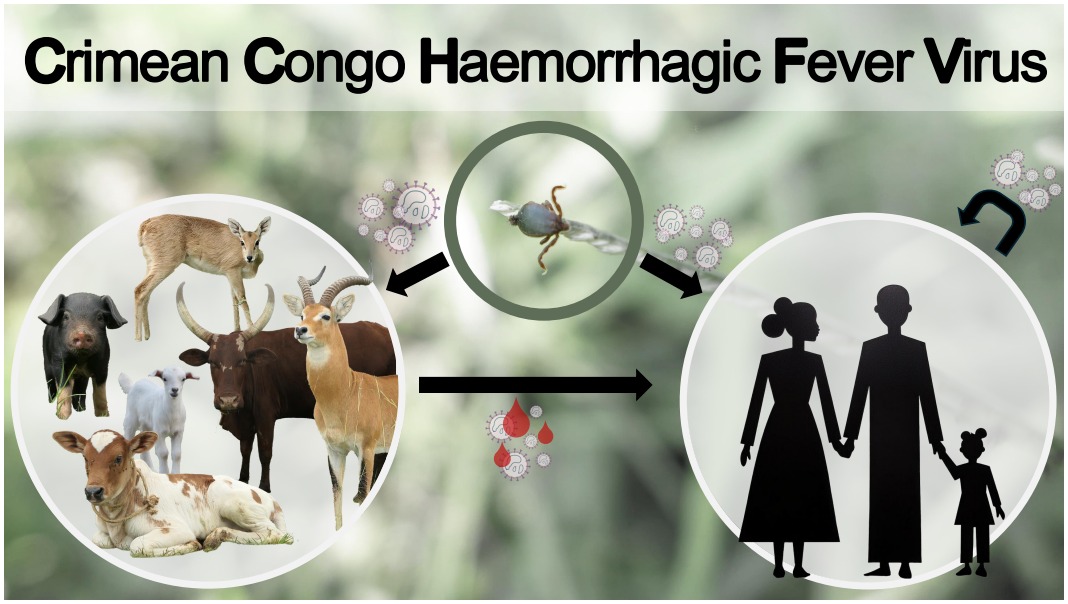

Figure 6.3: **Three Minute Thesis Competition.** Slide shows a simplified transmission cycle of CCHFV, which I used to present my PhD research within 3 minutes to a non-expert audience. All photographs were taken by me. The virus symbol is from Biorender, and the human family was created using the AI tool within Adobe Inc., 2025.

scientific audience at the Pint of Science event in Glasgow, 2025 (https://pintofscience.co.uk/events/glasgow). I was part of the session "Creative Explotions", where science meets art. For this event, I created a photo exhibition titled "Commonalities", aiming to highlight the importance of seeing the world as one and focusing on what unites us rather than what divides us. This message was especially meaningful to me, as my research on exposure risks for CCHFV primarily focused on identifying differences between regions and districts. Through photographs taken during my PhD travels, I sought to convey a sense of unity by pairing images that reflect shared elements. Sometimes the commonality is subtle, but with the right perspective, it can become clear. A picture of the exhibition is presented in Figure 6.4, and two examples are shown in Figure A.5 and A.6.

Figure 6.4: **Commonalities art exhibition** as part of the Pint of Science event in Glasgow

6.7 Conclusion

Community engagement helps bridge gaps and makes both our work and intentions better understood by the larger communities surrounding us. Vector Ludo engages children and young adults who are curious to experience something new and fun, and as a side effect, they read, see, and learn about vector-borne diseases and prevention strategies. The Three Minute Thesis Competition was an excellent opportunity to bring my research closer to academic audiences at universities worldwide. Finally, through the art exhibition, I found a meaningful way to interact with non-scientific audiences, including family and friends, about my research. These examples highlight just three of many tools that can be used to foster community engagement. I encourage more researchers to think about and get involved in community engagement.

Chapter 7

Discussion

This PhD explored mechanisms of human exposure to Crimean-Congo haemorrhagic fever virus (CCHFV) and other viral haemorrhagic fever viruses (VHFVs) in Uganda. By conducting and analysing multiple studies presented in this work, I sought to address gaps in existing knowledge and increase the understanding of community exposure to VHFVs in Uganda. Specifically, the research aimed to: (1) investigate risk factors associated with exposure to Ebola virus (EBOV), Rift Valley fever virus (RVFV) and CCHFV in healthcare workers (HCWs) compared to local communities, using data from a case-control serosurvey from five study sites in Uganda; (2) understand local and cultural differences associated with human-animal-tick interactions in six environmentally and socioecologically distinct districts of Uganda, by conducting focus group discussions (FGDs) and key informant interviews (KIIs); and (3) estimate exposure to CCHFV in four districts and analyse associated risk factors using a cross-sectional, household-based, randomised serosurvey.

7.1 Key findings

In the study described in Chapter 2, I aimed to estimate the seroprevalence of VHFVs in HCWs and community members in Uganda. A high seropositivity for CCHFV (19%) and EBOV (16%) was detected overall, while RVFV seropositivity was generally low (2%). Surprisingly, the highest odds of exposure were noted in Arua district for both EBOV (AOR = 9.01 [95% CI = 5.48-15.4]) and CCHFV (AOR = 4.67 [95% CI = 3.11-7.13]), an area with no recent documented case of VHFVs prior to study recruitment. Furthermore, contrary to the initial hypothesis that HCWs in Uganda are at significantly higher risk of exposure to VHFVs, mainly driven by occupational contact patterns, compared to members of the general community, the study found that HCWs had lower odds of seropositivity for both EBOV (AOR = 0.37, 95% CI = 0.26–0.51) and CCHFV (AOR = 0.42, 95% CI = 0.31–0.57) compared to matched local community members. Regarding occupational contact patterns, exploratory multivariable analysis found that homemakers and cleaners were the two occupational groups with the highest seropositivity for EBOV and CCHFV in the respective study groups (community members and HCWs). This indicates that local communities should be prioritised for intervention in future outbreaks, with a particular focus on those who are highly exposed but likely to be less educated about the risks of exposure.

In the study described in Chapter 3, I aimed to identify distinct environmental and socioecological areas within Uganda to study CCHFV exposure in representative areas in further studies. A subset of six districts was selected, based on notable differences in climate, land use, proximity to wildlife, and their subregional locations within Uganda.

In the qualitative survey of human-animal-tick interactions described in Chapter 4, I aimed to identify factors associated with exposure to CCHFV across six distinct districts in Uganda. Multiple risk factors were described, several

common behaviours shared across sites, as well as unique practices present only in a few. Ubiquitous exposure to tick bites and ticks was reported by participants in Kaabong, Soroti, Arua, and Kasese districts. Most participants identified children and young adults as being at the highest risk, mainly due to their roles in caring for animals. Animal cohabitation was reported in most districts, particularly in Kasese, where it was commonly attributed to fear of animal theft. Furthermore, the practice of slaughtering sick or dead animals was reported in most districts, except Kampala and Arua. A participant in the women's FGD in Kaabong stated that: *"we hardly slaughter animals for consumption unless they are sick, and they die"*. This highlights the regularity of slaughtering sick animals in Kaabong. Consuming fresh animal blood was also common in Kaabong and Kasese. In Kasese, participants mentioned ingesting fresh blood from guinea pigs to treat anaemia. In Arua and Soroti, blood was also reportedly consumed, but only after cooking. A historical practice in Kaabong, and still reported in Arua, is the collection and consumption of engorged ticks, collected directly from animals and then roasted. These findings on blood and tick consumption support the hypothesis that socioecological behaviours related to human-animal and human-tick interactions vary across Uganda, and are likely to be associated variably with exposure to zoonotic disease.

In Chapter 5, I found geographic heterogeneity in CCHFV seroprevalence across Uganda, as previously hypothesised. The estimated seroprevalence showed significant differences between Kasese (18.2%) and Soroti (2.8%), Kaabong (2.2%), and Kalangala (4.0%) districts ($p < 0.001$). Within-district variation in seroprevalence was only observed in Kalangala, which consists of many islands in Lake Victoria. Several potential risk behaviours for CCHFV exposure were recorded, including daily care for domestic animals (41.7%), having ever been bitten by a tick (34.7%), slaughtering or participating in the killing of animals (24.6%), consuming raw blood in the past year (6.5%), and having ever eaten a roasted tick (4.1%). These behaviours varied by district. Multivari-

able risk analysis identified district and age as the significant factors associated with CCHFV exposure. Following the full recruitment of additional districts, the dataset is likely to provide further insights to address the question of how differences in behaviour affect district-level seroprevalence. Seroprevalence increased with age, which was modelled through a force of infection model, indicating a constant rate of exposure and accumulation of seropositivity in older individuals. This is in keeping with an ongoing and under-estimated risk of CCHFV exposure in Uganda.

In Chapter 6, I introduced the Vector Ludo board game as a public engagement tool, designed for use in Uganda and other Sub-Saharan countries facing similar infectious disease burdens. The game is available in English and six local languages, and it has already been used to engage study participants and communities in our research areas. Vector Ludo facilitates conversations about disease vectors and the pathogens they transmit, including ticks and CCHFV. It can also be further integrated into more systematic educational programmes.

In summary, this PhD highlights the likely substantial burden of undetected disease associated with exposure to CCHFV in Uganda. This could be due to misdiagnosis of severe disease (Ashraf *et al.* 2025; Balinandi *et al.* 2024) or relatively mild infections that do not come to medical attention (Bodur *et al.* 2012). The high burden of CCHFV exposure was demonstrated by the findings in Chapter 2, which revealed an overall seroprevalence of 19% for CCHFV, as well as by the serosurvey in Chapter 5, which estimated a seroprevalence of 18.2% in Kasese district. The burden of CCHFV in Uganda is highly geographically heterogeneous, with the highest prevalence observed in Arua and Kasese districts, located in the North-Western and Western areas of Uganda. Heterogeneity was also evident within districts. For example, there was marked heterogeneity within Kalangala district, with high seroprevalence in one sampled village, suggesting the likelihood of specific local practices that may increase the risk of CCHFV exposure.

Homemakers in Arua, Gulu, and Kasese exhibited the highest seroprevalence for CCHFV (46/87; 52.9%), followed by farmers (48/213; 22.5%) and hospital cleaners (20/102; 19.6%). While the risk for farmers is well documented in literature (Atim *et al.* 2022; Hawman & Feldmann, 2023), the predominantly female group of homemakers has not been adequately considered in awareness campaigns or vaccination strategies. Women participating in the FGDs from Chapter 4 expressed appreciation for being included in discussions about CCHFV, noting that in Kalangala, they *"are the ones that do that work (caring for animals). Even if it is milking. I milk my own cow. Even taking it to the bushes to eat, I take it"*. This underscores the importance of incorporating local context in public health messaging and ensuring the participation of varied groups in local surveys. Many participants in the FGDs reported owning a few animals at home, which does not automatically classify them as farmers. Yet, their close contact with animals and activities such as grazing likely substantially increases their risk of tick bites and CCHFV exposure. The importance of including hospital cleaners in viral haemorrhagic fever (VHF) personal protective equipment (PPE) training and ensuring their access to appropriate PPE has previously been recognised (Cross *et al.* 2019; de la Fuente *et al.* 2024; Olu *et al.* 2015), but our study indicates that this issue requires more rigorous attention. These findings underscore the need for targeted interventions for these at-risk groups, including homemakers, women, and hospital cleaners.

The qualitative study in Chapter 4 highlights that participants predominantly believe children and young adults are at the highest risk for tick bites, compared to older adults. In our force of infection (FOI) model for CCHFV seroprevalence (Chapter 5), we assumed constant infection, with no antibody waning. While our dataset is not yet large enough to robustly model FOI by age, a trend emerges when seropositivity is plotted across age groups (Figure 5.11b). The increase in seroprevalence between ages 2-14 and 15-27 is minimal (+0.02%) compared to the initial exposure in the youngest group

(1.89%). In contrast, the increase between ages 15-27 and 28-40 is +4.0%, and between 28-40 and those 41 and above is +5.46%. Future research is needed to accurately determine age-specific FOI, but our findings indicate that exposure occurs across all age groups, including young children as well as older adults.

7.2 Limitations

Overall, this PhD had a few key limitations. Firstly, we used two different ELISA assays (VectorBest and IDScreen) for CCHFV antibody detection in the HCWs serosurvey and the general serosurvey, due to the unavailability of VectorBest after 2022. It is therefore challenging to directly compare seropositivity results between the studies. The assays consist of different antigens (whole virus vs. NP protein), strains of the virus, and conjugate systems. Their reported sensitivity and specificity vary slightly, and specific data for human samples from Uganda are unavailable. Our validation experiment of the IDScreen assay with 10 convalescent Ugandan patients detected all samples as positive, however larger experiments would provide future additional reassurance.

Secondly, cross-reactivity to related nairoviruses has been reported for animal samples (Atim, Niebel, *et al.* 2023; Maze *et al.* 2025) and needs further investigation in human populations (Karaaslan *et al.* 2025). Multiple nairoviruses circulate in Uganda, including Dugbe virus and Nairobi sheep disease virus (Atim, Ashraf, *et al.* 2023). There is a high potential for cross-reactivity following exposure to orthonairoviruses when testing for CCHFV by serology, as reported in domestic animal cohorts by Atim *et al.* 2022; Maze *et al.* 2025. Further work with human samples is needed to better distinguish CCHFV exposure from possible cross-reactivity due to the assays, although reports of human disease with Dugbe virus and NSDV are exceptionally rare.

Additionally, detected antibodies against CCHFV, EBOV, or RVFV may not always reflect the actual burden of exposure, due to antibody waning or individuals not developing antibody responses (Shepherd *et al.* 1989). Interestingly, the first description of reinfection of CCHFV has been reported by Buyuktuna *et al.* 2026, challenging the dogma of life-long protection, which should be considered further in future serosurvey analyses and FOI models. Host factors are additionally likely to influence seropositivity, such as coinfections with helminths or HIV, as well as polymorphisms in innate and adaptive immune responses (Rao *et al.* 2025).

Another key challenge across all studies described in this PhD was the diversity of local languages spoken in Uganda and at our study sites. This linguistic variation may have introduced bias, such as slight differences in translation by the study teams during conduct of FGDs, KIIs, or in recording risk behaviours in the seroprevalence surveys. To mitigate this, we worked with well-trained study teams, invited team leads to a training day in Entebbe to harmonise recruitment procedures for Chapter 5, and followed clear instructions and protocols.

Both the seroprevalence and qualitative studies have inherent limitations. As outlined in the introduction, I argue that combining both in a mixed methods approach enhanced the overall quality and depth of the findings. The limitations of the qualitative study in Chapter 4, such as the inability to generalise or quantify exposure risk, were addressed by the quantitative study in Chapter 5, which provided estimated seroprevalence for the sampled districts. Conversely, the typical lack of contextual depth in serosurveys was mitigated by the detailed exploration of human-animal-tick interactions through FGDs and KIIs (Chapter 4). A more deeply integrated mixed methods approach, involving multiple engagements with communities before, during and after a serosurvey, would further strengthen research of this kind.

A One Health approach is essential to understand a zoonotic virus like CCHFV.

We have not yet reached the point in the AVI study to integrate data from animals and ticks into risk-based analyses in humans. However, at a later date, the team will test domestic animals from the same households for exposure to orthonairoviruses, as well as collect ticks from the animals and the environment in the villages, for genomic material of CCHFV and other orthonairoviruses. This will help answer questions related to transmission risks and clustering of exposure.

Ideally, we would have investigated the highlighted exposure risks for CCHFV across all 13 distinct clusters identified by the K-prototype analysis in Chapter 3. However, due to time and financial constraints, this was not feasible within the scope of this PhD. Future work within the Thomson group will address this gap.

7.3 Recommendations and future research

Building on my key findings from this PhD, I propose the following recommendations and areas of future research to increase the knowledge around CCHFV exposure in Uganda and beyond:

- Further research is needed to better understand the role of environmental and land use factors in CCHFV transmission. Significant differences in seroprevalence between Kasese and other districts can not fully be explained by risk practices alone. Environmental conditions that support tick presence, as well as land use patterns, such as the movement of ticks between wild and domestic animals, should be investigated using spatial modelling tools. This includes more detailed tick surveys and experimental studies to understand the role of the different tick species in the transmission of CCHFV.

- Interventions for CCHFV should be investigated in Uganda with the lo-

cal context in mind. That is true for all countries and regions where CCHFV is present. Our findings showed that human-animal-tick interactions and exposure to CCHFV are common and often context-specific, warranting thorough investigation. This should include engagement with local leaders and community members to identify potential interventions that could reduce the burden of CCHFV.

- Understanding the biological risks associated with reported practices can help map their relevance to CCHFV transmission. This is particularly important for tick exposure and blood consumption, as there is limited knowledge about the infectious dose required for transmission and how different routes of exposure affect infection likelihood. Research should also explore the durability of CCHFV, especially in slaughtered infected animals and their products. Only then can we use our results and inform communities about their risk of CCHFV transmission through specific practices with scientific evidence.

- Investigations into antibodies directed against CCHFV in humans are needed. This should include studies on cross-reactivity with other nairoviruses, avidity and impact on seroprevalence using different assays, neutralisation using live virus or pseudoparticle systems, antibody longevity, and how host factors influence protection for CCHFV.

- Incorporating qualitative components into quantitative research should become standard in infectious disease epidemiology. A mixed-methods approach can provide contextual insights for public health strategies. This aligns with efforts to decolonise global health by amplifying the perspectives of those most affected by local diseases.

- This PhD thesis presents an approach developed and implemented in Uganda, which could be adapted to other countries. CCHFV is present in multiple countries across Africa, Europe and Asia, and context-specific, systematic serosurveys could support education and control efforts in all

of these regions. The approach outlined here for CCHFV in Uganda is particularly relevant for regions where tick-borne pathogens are emerging due to climate change, and where contextualised risk assessments have not yet been conducted, such as the introduction of tick-borne encephalitis virus in the United Kingdom (Holding *et al.* 2020), or CCHFV to countries like Austria and Germany (Fanelli & Buonavoglia, 2021).

7.4 Positionality and personal reflection

As a researcher with a background in molecular medicine and biomedical science, specialising in infectious and tropical diseases, I began this PhD knowing there was much for me to learn.

I have been studying, working, and travelling within Uganda for weeks or months at a time since 2015. This journey began with two semesters spent at Makerere University in Kampala. Being born in Germany, in the Global North, placed me in a privileged situation to afford these opportunities, but I also recognised that with privilege comes responsibility. This awareness has shaped my research career, my PhD topic, and my overall approach to life.

Although I had no prior training in social science, I was eager to incorporate methodologies into my PhD that could capture local context and include the communities affected by the disease. This influenced the planning, execution and analysis of FGDs and KIIs. I believe my motivation was genuine, and the training I received was valuable. I relied on local scientists who spoke the local languages to engage with communities, which helped open doors and navigate the complexities of power dynamics simultaneously. These dynamics are especially present in infectious disease research conducted far from home.

This PhD has brought significant personal growth and placed me at the starting point of a career in viral disease ecology. I intend to progress in the fol-

lowing year, funded by the Wellcome Trust, with improving my mathematical modelling skills at Oregon State University in the United States.

7.5 Conclusions

This PhD has contributed to a deeper understanding of CCHFV seroprevalence and risk in Uganda by integrating serosurveys with qualitative and ecological data. It has highlighted the complexity of CCHFV transmission and the importance of context-specific interventions. The work has added to the increasing knowledge developed over the recent years and serves as a foundation for continued exploration into viral disease ecology, with the aim of reducing the burden of infectious diseases in Uganda and globally.

Appendix A

Additional tables and figures

This contains a summary of additional tables and documents, which help to see the full picture of the thesis work.

	Healthcare worker (N=639)	Community member (N=714)	Total (N=1353)
Subregion			
Acholi	161 (25.2%)	151 (21.1%)	312 (23.1%)
West Nile	178 (27.9%)	128 (17.9%)	306 (22.6%)
Western (Bwera)	150 (23.5%)	222 (31.1%)	372 (27.5%)
Western (Kagando)	150 (23.5%)	213 (29.8%)	363 (26.8%)
Sex			
Female	416 (65.1%)	485 (67.9%)	901 (66.6%)
Male	223 (34.9%)	229 (32.1%)	452 (33.4%)
Age			
Mean (SD)	35.2 (11.1)	32.6 (11.5)	33.8 (11.4)
Median [Min, Max]	34.0 [18.0, 74.0]	30.0 [18.0, 77.0]	32.0 [18.0, 77.0]
Age (years)			
18 to 27	204 (31.9%)	304 (42.6%)	508 (37.5%)
28 to 37	170 (26.6%)	200 (28.0%)	370 (27.3%)
38 to 47	154 (24.1%)	116 (16.2%)	270 (20.0%)
48 to 57	89 (13.9%)	66 (9.2%)	155 (11.5%)
58 to 77	22 (3.4%)	28 (3.9%)	50 (3.7%)
rVSV-ZEBOV-GP vaccine			
Yes	181 (28.3%)	2 (0.3%)	183 (13.5%)
No	453 (70.9%)	555 (77.7%)	1008 (74.5%)
Not recorded	5 (0.8%)	157 (22.0%)	162 (12.0%)
Hepatitis B vaccination			
Full vaccinated (2 doses)	565 (88.4%)	187 (26.2%)	752 (55.6%)
Unvaccinated	53 (8.3%)	211 (29.6%)	264 (19.5%)
Partly vaccinated (1 dose)	7 (1.1%)	7 (1.0%)	14 (1.0%)
Not recorded	14 (2.2%)	309 (43.3%)	323 (23.9%)
Housing condition			
Permanent	480 (75.1%)	243 (34.0%)	723 (53.4%)
Semi-permanent	98 (15.3%)	259 (36.3%)	357 (26.4%)
Temporary	59 (9.2%)	203 (28.4%)	262 (19.4%)
Not recorded	2 (0.3%)	9 (1.3%)	11 (0.8%)
Travelled away from home in the past three weeks			
Yes	128 (20.0%)	69 (9.7%)	197 (14.6%)
No	509 (79.7%)	643 (90.1%)	1152 (85.1%)
Not recorded	2 (0.3%)	2 (0.3%)	4 (0.3%)
Visited caves or mines within the last three weeks			
Yes	9 (1.4%)	5 (0.7%)	14 (1.0%)
No	626 (98.0%)	709 (99.3%)	1335 (98.7%)
Not recorded	4 (0.6%)	0 (0%)	4 (0.3%)
Exposure to VHF case			
Yes	20 (3.1%)	18 (2.5%)	38 (2.8%)
No	593 (92.8%)	568 (79.6%)	1161 (85.8%)
Not recorded	26 (4.1%)	128 (17.9%)	154 (11.4%)
Tick bite in the past 3 weeks			
Yes	4 (0.6%)	10 (1.4%)	14 (1.0%)
No	635 (99.4%)	704 (98.6%)	1339 (99.0%)
Mosquito bite in the past 3 weeks			
Yes	545 (85.3%)	559 (78.3%)	1104 (81.6%)
No	94 (14.7%)	155 (21.7%)	249 (18.4%)
Bats roosting in the home, close to the home or at the place of work			
Yes	163 (25.5%)	209 (29.3%)	372 (27.5%)
No	469 (73.4%)	493 (69.0%)	962 (71.1%)
Not recorded	7 (1.1%)	12 (1.7%)	19 (1.4%)
Rodents or evidence of rodents in the house			
Yes	415 (64.9%)	523 (73.2%)	938 (69.3%)
No	223 (34.9%)	188 (26.3%)	411 (30.4%)
Not recorded	1 (0.2%)	3 (0.4%)	4 (0.3%)
Care for mammals			
Yes	279 (43.7%)	452 (63.3%)	731 (54.0%)
No	360 (56.3%)	262 (36.7%)	622 (46.0%)
Animals you care for died unexpectedly in the past month			
Yes	24 (3.8%)	30 (4.2%)	54 (4.0%)
No	611 (95.6%)	679 (95.1%)	1290 (95.3%)
Not recorded	4 (0.6%)	5 (0.7%)	9 (0.7%)
Killed or butchered an animal in the last month			
Yes	68 (10.6%)	75 (10.5%)	143 (10.6%)
No	569 (89.0%)	634 (88.8%)	1203 (88.9%)
Not recorded	2 (0.3%)	5 (0.7%)	7 (0.5%)
Contact with wild animal in the last month			
Yes	13 (2.0%)	14 (2.0%)	27 (2.0%)
No	623 (97.5%)	689 (96.5%)	1312 (97.0%)
Not recorded	3 (0.5%)	11 (1.5%)	14 (1.0%)

Table A.1: Full demographic data of participants by study group (HCW study Chapter 2).

a)

Healthcare worker occupations	EBOV positive [ratio (%)]	CCHFV positive [ratio (%)]	RVFV positive [ratio (%)]
Nurse	29/270 (10.7%)	30/270 (11.1%)	3/202 (1.5%)
Cleaner	23/102 (22.5%)	20/102 (19.6%)	1/77 (1.3%)
Midwifery	5/85 (5.9%)	14/85 (16.5%)	1/81 (1.2%)
Doctor	5/52 (9.6%)	3/52 (5.8%)	0/34
Laboratory personal	6/32 (18.8%)	2/32 (6.2%)	0/23
Other occupations within the hospital	5/27 (18.5%)	9/27 (33.3%)	2/15 (13.3%)
Administrative	0/24	4/24 (16.7%)	0/20
Facilities	1/12 (8.3%)	1/12 (8.3%)	0/11
Mental health worker	0/12	1/12 (8.3%)	0/7
Security	1/11 (9.1%)	3/11 (27.3%)	0/11
Mortuary worker	2/9 (22.2%)	2/9 (22.2%)	0/3
Village Health Team (VHT)	0/3	0/3	0/3

b)

Community member occupations	EBOV positive [ratio (%)]	CCHFV positive [ratio (%)]	RVFV positive [ratio (%)]
Farming and animal handling	40/213 (18.8%)	48/213 (22.5%)	3/173 (1.7%)
Business	18/156 (11.5%)	26/157 (16.6%)	4/144 (2.8%)
Casual work	13/93 (14%)	17/93 (18.3%)	2/85 (2.4%)
Homemaker	39/87 (44.8%)	46/87 (52.9%)	0/60
Education	15/80 (18.8%)	9/80 (11.2%)	1/78 (1.3%)
No occupation	10/28 (35.7%)	15/28 (53.6%)	0/20
Transport	3/26 (11.5%)	1/26 (3.8%)	1/23 (4.3%)
Craftsmanship	4/24 (16.7%)	2/24 (8.3%)	1/22 (4.5%)
Security	1/4 (25%)	2/4 (50%)	0/2
Religion	1/2 (50%)	1/2 (50%)	0/2

Table A.2: Seropositivity of VHFV for different occupational groups for (a) HCWs and (b) Community members (HCW study Chapter 2).

Characteristic	EBOV positive, N = 221[1]	EBOV negative, N = 1,131[1]	Univariable regression			Multivariable regression		
			OR[2]	95% CI	Univariable p.value	AOR[3]	95% CI	Multivariable p.value
Study group					**<0.001**			**<0.001**
HCW	77 (12.1%)	562 (87.9%)	0.54	0.40, 0.73		0.37	0.26, 0.51	
Community member	144 (20.2%)	569 (79.8%)	—	—		—	—	
Study location					**<0.001**			**<0.001**
Kasese district (Bwera)	25 (6.7%)	347 (93.3%)	—	—		—	—	
Kasese district (Kagando)	43 (11.9%)	319 (88.1%)	1.87	1.13, 3.17		2.19	1.27, 3.86	
Arua district	96 (31.4%)	210 (68.6%)	6.35	4.02, 10.4		9.01	5.48, 15.4	
Gulu district	57 (18.3%)	255 (81.7%)	3.10	1.91, 5.18		4.15	2.43, 7.31	
Sex					0.086			**0.008**
Male	85 (18.8%)	367 (81.2%)	1.30	0.96, 1.75		1.57	1.13, 2.17	
Female	136 (15.1%)	764 (84.9%)	—	—		—	—	
Age					0.3			0.8
18 to 27	74 (14.6%)	433 (85.4%)	—	—		—	—	
28 to 37	58 (15.7%)	312 (84.3%)	1.09	0.75, 1.58		0.97	0.64, 1.45	
38 to 47	54 (20.0%)	216 (80.0%)	1.46	0.99, 2.15		1.27	0.82, 1.95	
48 to 57	24 (15.5%)	131 (84.5%)	1.07	0.64, 1.75		1.00	0.57, 1.71	
58 to 77	11 (22.0%)	39 (78.0%)	1.65	0.77, 3.27		1.04	0.45, 2.25	
Visited caves or mines within the last three weeks[4]					0.2			
Yes	4 (28.6%)	10 (71.4%)	2.07	0.56, 6.25				
No	216 (16.2%)	1,118 (83.8%)	—	—				
Contact with wild animal in the last month					0.4			0.2
Yes	3 (11.1%)	24 (88.9%)	0.64	0.15, 1.84		0.43	0.10, 1.32	
No	215 (16.4%)	1,096 (83.6%)	—	—		—	—	
Bats roosting in the home, close to the home or at the place of work					**0.005**			0.14
Yes	78 (21.0%)	294 (79.0%)	1.56	1.14, 2.11		1.30	0.92, 1.81	
No	140 (14.6%)	821 (85.4%)	—	—		—	—	
Ever exposed to a VHFV[5]					0.050			
Yes	9 (23.7%)	29 (76.3%)	2.28	1.00, 4.73				
No	139 (12.0%)	1,021 (88.0%)	—	—				

[1] n (%)

[2] OR = Odds Ratio

[3] AOR = Adjusted Odds Ratio

[4] removed from multivariable regression due to less than 15 reports in risk category

[5] removed from multivariable regression due to 154 'Don't know' values

Table A.3: Univariable and multivariable regression analysis of EBOV seropositivity (HCW study Chapter 2).

Characteristic	EBOV positive, N = 221[1]	EBOV negative, N = 1,131[1]	Univariable regression			Multivariable regression		
			OR[2]	95% CI	Univariable p.value	AOR[3]	95% CI	Multivariable p.value
Study group					**<0.001**			**0.018**
HCW	77 (12.1%)	562 (87.9%)	0.54	0.40, 0.73		0.63	0.42, 0.92	
Community member	144 (20.2%)	569 (79.8%)	—	—		—	—	
Study location					**<0.001**			**<0.001**
Kasese district (Bwera)	25 (6.7%)	347 (93.3%)	—	—		—	—	
Kasese district (Kagando)	43 (11.9%)	319 (88.1%)	1.87	1.13, 3.17		1.94	1.12, 3.42	
Arua district	96 (31.4%)	210 (68.6%)	6.35	4.02, 10.4		4.96	2.89, 8.73	
Gulu district	57 (18.3%)	255 (81.7%)	3.10	1.91, 5.18		2.00	1.08, 3.78	
Sex					0.086			**0.002**
Male	85 (18.8%)	367 (81.2%)	1.30	0.96, 1.75		1.73	1.23, 2.43	
Female	136 (15.1%)	764 (84.9%)	—	—		—	—	
Visited caves or mines within the last three weeks					0.2			
Yes	4 (28.6%)	10 (71.4%)	2.07	0.56, 6.25				
No	216 (16.2%)	1,118 (83.8%)	—	—				
Contact with wild animal in the last month					0.4			
Yes	3 (11.1%)	24 (88.9%)	0.64	0.15, 1.84				
No	215 (16.4%)	1,096 (83.6%)	—	—				
Bats roosting in the home, close to the home or at the place of work					**0.005**			0.2
Yes	78 (21.0%)	294 (79.0%)	1.56	1.14, 2.11		1.29	0.91, 1.83	
No	140 (14.6%)	821 (85.4%)	—	—		—	—	
Ever exposed to a VHFV[4]					0.050			
Yes	9 (23.7%)	29 (76.3%)	2.28	1.00, 4.73				
No	139 (12.0%)	1,021 (88.0%)	—	—				
Age [median (IQR)]	33 (25, 43)	31 (24, 41)	1.01	1.00, 1.02	0.075			
Age					0.3			0.9
18 to 27	74 (14.6%)	433 (85.4%)	—	—		—	—	
28 to 37	58 (15.7%)	312 (84.3%)	1.09	0.75, 1.58		1.06	0.70, 1.59	
38 to 47	54 (20.0%)	216 (80.0%)	1.46	0.99, 2.15		1.25	0.80, 1.94	
48 to 57	24 (15.5%)	131 (84.5%)	1.07	0.64, 1.75		1.01	0.57, 1.75	
58 to 77	11 (22.0%)	39 (78.0%)	1.65	0.77, 3.27		1.26	0.55, 2.68	
Housing condition					**<0.001**			**<0.001**
Permanent	77 (10.7%)	646 (89.3%)	0.76	0.52, 1.13		0.79	0.51, 1.25	
Semi-permanent	48 (13.5%)	308 (86.5%)	—	—		—	—	
Temporary	94 (35.9%)	168 (64.1%)	3.59	2.43, 5.36		2.36	1.38, 4.07	
Travelled away from home in the past three weeks					0.9			
Yes	33 (16.8%)	164 (83.2%)	1.03	0.68, 1.53				
No	188 (16.3%)	963 (83.7%)	—	—				
Care for domestic animals					**0.020**			0.7
Yes	135 (18.5%)	595 (81.5%)	1.41	1.06, 1.90		0.94	0.67, 1.33	
No	86 (13.8%)	536 (86.2%)	—	—		—	—	
Animals you care for died unexpectedly in the past month					0.064			0.6
Yes	14 (25.9%)	40 (74.1%)	1.86	0.96, 3.40		1.22	0.60, 2.35	
No	204 (15.8%)	1,085 (84.2%)	—	—		—	—	
Killed or butchered an animal in the last month					0.3			
Yes	19 (13.3%)	124 (86.7%)	0.76	0.45, 1.24				
No	201 (16.7%)	1,001 (83.3%)	—	—				
Tick bite in the past 3 weeks[5]					0.078			
Yes	5 (35.7%)	9 (64.3%)	2.89	0.88, 8.44				
No	216 (16.1%)	1,122 (83.9%)	—	—				
Mosquito bite in the past 3 weeks					**0.003**			**0.019**
Yes	164 (14.9%)	939 (85.1%)	0.59	0.42, 0.83		0.62	0.42, 0.92	
No	57 (22.9%)	192 (77.1%)	—	—		—	—	
Rodents or evidence of rodents in the house					0.4			
Yes	148 (15.8%)	790 (84.2%)	0.86	0.64, 1.18				
No	73 (17.8%)	337 (82.2%)	—	—				
rVSV-ZEBOV-GP vaccine					0.2			
Yes	24 (13.1%)	159 (86.9%)	—	—				
No	167 (16.6%)	840 (83.4%)	1.32	0.85, 2.13				

[1] n (%); Median (IQR)

[2] OR = Odds Ratio

[3] AOR = Adjusted Odds Ratio

[4] removed from multivariable regression due to 154 'Don't know' values

[5] removed from multivariable regression due to less than 15 reports in risk category

Table A.4: Exploratory logistic regression analysis of all variables for EBOV seropositivity risk (HCW study Chapter 2).

Characteristic	CCHFV positive, N = 256[1]	CCHFV negative, N = 1,097[1]	Univariable regression			Multivariable regression		
			OR[2]	95% CI	Univariable p.value	AOR[3]	95% CI	Multivariable p.value
Study group					**<0.001**			**<0.001**
HCW	89 (13.9%)	550 (86.1%)	0.53	0.40, 0.70		0.42	0.31, 0.57	
Community member	167 (23.4%)	547 (76.6%)	—	—		—	—	
Study location					**<0.001**			**<0.001**
Kasese district (Bwera)	43 (11.6%)	329 (88.4%)	—	—		—	—	
Kasese district (Kagando)	67 (18.5%)	296 (81.5%)	1.73	1.15, 2.63		1.86	1.22, 2.87	
Arua district	105 (34.3%)	201 (65.7%)	4.00	2.71, 5.99		4.67	3.11, 7.13	
Gulu district	41 (13.1%)	271 (86.9%)	1.16	0.73, 1.83		1.24	0.77, 1.99	
Sex					>0.9			>0.9
Male	85 (18.8%)	367 (81.2%)	0.99	0.74, 1.32		1.01	0.74, 1.38	
Female	171 (19.0%)	730 (81.0%)	—	—		—	—	
Age					**0.003**			**0.002**
18 to 27	86 (16.9%)	422 (83.1%)	—	—		—	—	
28 to 37	61 (16.5%)	309 (83.5%)	0.97	0.67, 1.39		1.00	0.69, 1.46	
38 to 47	65 (24.1%)	205 (75.9%)	1.56	1.08, 2.23		1.74	1.17, 2.58	
48 to 57	26 (16.8%)	129 (83.2%)	0.99	0.60, 1.58		1.09	0.65, 1.81	
58 to 77	18 (36.0%)	32 (64.0%)	2.76	1.46, 5.10		2.95	1.48, 5.75	
Killed or butchered an animal in the last month					0.2			0.3
Yes	21 (14.7%)	122 (85.3%)	0.71	0.43, 1.13		0.77	0.45, 1.26	
No	234 (19.5%)	969 (80.5%)	—	—		—	—	
Tick bite in the past 3 weeks[4]					**0.039**			
Yes	6 (42.9%)	8 (57.1%)	3.27	1.07, 9.48				
No	250 (18.7%)	1,089 (81.3%)	—	—				
Ever exposed to a VHFV[5]					0.5			
Yes	7 (18.4%)	31 (81.6%)	1.38	0.55, 3.02				
No	163 (14.0%)	998 (86.0%)	—	—				

[1] n (%)

[2] OR = Odds Ratio

[3] AOR = Adjusted Odds Ratio

[4] removed from multivariable regression due to less than 15 reports in risk category

[5] removed from multivariable regression due to 154 'Don't know' values

Table A.5: Univariable and multivariable logistic regression analysis of CCHFV seropositivity (HCW study Chapter 2).

Characteristic	CCHFV positive, N = 256[1]	CCHFV negative, N = 1,097[1]	Univariable regression			Multivariable regression		
			OR[2]	95% CI	Univariable p.value	AOR[3]	95% CI	Multivariable p.value
Study group					**<0.001**			**<0.001**
HCW	89 (13.9%)	550 (86.1%)	0.53	0.40, 0.70		0.54	0.38, 0.77	
Community member	167 (23.4%)	547 (76.6%)	—	—		—	—	
Study location					**<0.001**			**<0.001**
Kasese district (Bwera)	43 (11.6%)	329 (88.4%)	—	—		—	—	
Kasese district (Kagando)	67 (18.5%)	296 (81.5%)	1.73	1.15, 2.63		1.98	1.27, 3.11	
Arua district	105 (34.3%)	201 (65.7%)	4.00	2.71, 5.99		3.32	2.06, 5.40	
Gulu district	41 (13.1%)	271 (86.9%)	1.16	0.73, 1.83		0.74	0.41, 1.33	
Sex					>0.9			0.9
Male	85 (18.8%)	367 (81.2%)	0.99	0.74, 1.32		1.03	0.75, 1.41	
Female	171 (19.0%)	730 (81.0%)	—	—		—	—	
Killed or butchered an animal in the last month					0.2			
Yes	21 (14.7%)	122 (85.3%)	0.71	0.43, 1.13				
No	234 (19.5%)	969 (80.5%)	—	—				
Tick bite in the past 3 weeks[4]					**0.039**			
Yes	6 (42.9%)	8 (57.1%)	3.27	1.07, 9.48				
No	250 (18.7%)	1,089 (81.3%)	—	—				
Ever exposed to a VHFV					0.5			
Yes	7 (18.4%)	31 (81.6%)	1.38	0.55, 3.02				
No	163 (14.0%)	998 (86.0%)	—	—				
Visited caves or mines within the last three weeks					0.2			
Yes	1 (7.1%)	13 (92.9%)	0.33	0.02, 1.65				
No	254 (19.0%)	1,081 (81.0%)	—	—				
Contact with wild animal in the last month					0.6			
Yes	4 (14.8%)	23 (85.2%)	0.74	0.22, 1.94				
No	250 (19.1%)	1,062 (80.9%)	—	—				
Bats roosting in the home, close to the home or at the place of work					0.4			
Yes	65 (17.5%)	307 (82.5%)	0.87	0.63, 1.18				
No	188 (19.5%)	774 (80.5%)	—	—				
Age [median (IQR)]	34 (25, 44)	31 (24, 41)	1.02	1.01, 1.03	**0.002**			
Age					**0.003**			**0.007**
18 to 27	86 (16.9%)	422 (83.1%)	—	—		—	—	
28 to 37	61 (16.5%)	309 (83.5%)	0.97	0.67, 1.39		1.00	0.68, 1.46	
38 to 47	65 (24.1%)	205 (75.9%)	1.56	1.08, 2.23		1.67	1.11, 2.52	
48 to 57	26 (16.8%)	129 (83.2%)	0.99	0.60, 1.58		1.10	0.64, 1.84	
58 to 77	18 (36.0%)	32 (64.0%)	2.76	1.46, 5.10		2.83	1.40, 5.59	
Housing condition					**<0.001**			**<0.001**
Permanent	109 (15.1%)	614 (84.9%)	0.97	0.69, 1.39		1.36	0.92, 2.01	
Semi-permanent	55 (15.4%)	302 (84.6%)	—	—		—	—	
Temporary	89 (34.0%)	173 (66.0%)	2.82	1.93, 4.17		2.97	1.76, 5.06	
Travelled away from home in the past three weeks					**0.035**			0.074
Yes	27 (13.7%)	170 (86.3%)	0.64	0.41, 0.97		0.66	0.41, 1.04	
No	229 (19.9%)	923 (80.1%)	—	—		—	—	
Care for domestic animals					**<0.001**			0.4
Yes	162 (22.2%)	569 (77.8%)	1.60	1.21, 2.12		1.16	0.85, 1.60	
No	94 (15.1%)	528 (84.9%)	—	—		—	—	
Animals you care for died unexpectedly in the past month					>0.9			
Yes	10 (18.5%)	44 (81.5%)	0.98	0.46, 1.89				
No	243 (18.8%)	1,047 (81.2%)	—	—				
Mosquito bite in the past 3 weeks					0.056			0.4
Yes	198 (17.9%)	906 (82.1%)	0.72	0.52, 1.01		0.87	0.60, 1.26	
No	58 (23.3%)	191 (76.7%)	—	—		—	—	
Rodents or evidence of rodents in the house					0.4			
Yes	172 (18.3%)	766 (81.7%)	0.89	0.66, 1.19				
No	83 (20.2%)	328 (79.8%)	—	—				

[1] n (%); Median (IQR)

[2] OR = Odds Ratio

[3] AOR = Adjusted Odds Ratio

[4] removed from multivariable regression due to less than 15 reports in risk category

Table A.6: Exploratory analysis of variables associated with CCHFV seropositivity (HCW study Chapter 2).

Characteristic	RVFV positive, N = 19[1]	RVFV negative, N = 1,077[1]	Univariable regression			Multivariable regression		
			OR[2]	95% CI	Univariable p.value	AOR[3]	95% CI	Multivariable p.value
Study group					0.5			0.5
HCW	7 (1.4%)	480 (98.6%)	0.73	0.27, 1.82		0.71	0.26, 1.82	
Community member	12 (2.0%)	597 (98.0%)	—	—		—	—	
Study location					>0.9			>0.9
Kasese district (Bwera)	6 (1.6%)	366 (98.4%)	—	—		—	—	
Kasese district (Kagando)	6 (1.7%)	343 (98.3%)	1.07	0.33, 3.44		1.15	0.35, 3.79	
Arua district	3 (1.7%)	172 (98.3%)	1.06	0.22, 4.08		0.90	0.18, 3.68	
Gulu district	4 (2.0%)	196 (98.0%)	1.24	0.32, 4.41		0.96	0.21, 3.81	
Sex					0.8			0.6
Male	7 (1.9%)	361 (98.1%)	1.16	0.43, 2.90		1.32	0.48, 3.41	
Female	12 (1.6%)	716 (98.4%)	—	—		—	—	
Age					0.6			0.6
18 to 27	6 (1.3%)	444 (98.7%)	—	—		—	—	
28 to 37	7 (2.4%)	286 (97.6%)	1.81	0.60, 5.68		1.82	0.59, 5.79	
38 to 47	4 (2.1%)	184 (97.9%)	1.61	0.41, 5.70		1.62	0.40, 5.96	
48 to 57	2 (1.6%)	120 (98.4%)	1.23	0.18, 5.43		1.27	0.18, 5.84	
58 to 77	0 (0.0%)	43 (100.0%)	0.00			0.00		
Killed or butchered an animal in the last month					0.5			0.6
Yes	3 (2.7%)	109 (97.3%)	1.65	0.38, 5.06		1.44	0.32, 4.69	
No	16 (1.6%)	961 (98.4%)	—	—		—	—	
Animals you care for died unexpectedly in the past month					0.2			0.2
Yes	2 (5.4%)	35 (94.6%)	3.48	0.54, 12.8		3.51	0.50, 15.8	
No	17 (1.6%)	1,034 (98.4%)	—	—		—	—	
Mosquito bite in the past 3 weeks					0.7			0.8
Yes	15 (1.7%)	882 (98.3%)	0.83	0.30, 2.93		0.83	0.29, 3.01	
No	4 (2.0%)	195 (98.0%)	—	—		—	—	

[1] n (%)

[2] OR = Odds Ratio

[3] AOR = Adjusted Odds Ratio

Table A.7: Univariable and multivariable logistic regression analysis of RVFV seropositivity (HCW study Chapter 2).

Characteristic	RVFV positive, N = 19[1]	RVFV negative, N = 1,077[1]	Univariable regression			Multivariable regression		
			OR[2]	95% CI	Univariable p.value	AOR[3]	95% CI	Multivariable p.value
Study group					0.5			0.6
HCW	7 (1.4%)	480 (98.6%)	0.73	0.27, 1.82		0.77	0.28, 1.95	
Community member	12 (2.0%)	597 (98.0%)	—	—		—	—	
Study location					>0.9			>0.9
Kasese district (Bwera)	6 (1.6%)	366 (98.4%)	—	—		—	—	
Kasese district (Kagando)	6 (1.7%)	343 (98.3%)	1.07	0.33, 3.44		1.03	0.32, 3.33	
Arua district	3 (1.7%)	172 (98.3%)	1.06	0.22, 4.08		1.23	0.25, 4.85	
Gulu district	4 (2.0%)	196 (98.0%)	1.24	0.32, 4.41		1.23	0.30, 4.47	
Sex					0.8			0.6
Male	7 (1.9%)	361 (98.1%)	1.16	0.43, 2.90		1.31	0.48, 3.38	
Female	12 (1.6%)	716 (98.4%)	—	—		—	—	
Killed or butchered an animal in the last month					0.5			
Yes	3 (2.7%)	109 (97.3%)	1.65	0.38, 5.06				
No	16 (1.6%)	961 (98.4%)	—	—				
Animals you care for died unexpectedly in the past month					0.2			
Yes	2 (5.4%)	35 (94.6%)	3.48	0.54, 12.8				
No	17 (1.6%)	1,034 (98.4%)	—	—				
Mosquito bite in the past 3 weeks					0.7			
Yes	15 (1.7%)	882 (98.3%)	0.83	0.30, 2.93				
No	4 (2.0%)	195 (98.0%)	—	—				
Ever exposed to a VHFV[4]					0.059			
Yes	2 (9.1%)	20 (90.9%)	6.06	0.92, 23.3				
No	16 (1.6%)	970 (98.4%)	—	—				
Visited caves or mines within the last three weeks					0.6			
Yes	0 (0.0%)	8 (100.0%)	0.00					
No	19 (1.8%)	1,065 (98.2%)	—	—				
Contact with wild animal in the last month					0.4			
Yes	0 (0.0%)	18 (100.0%)	0.00					
No	19 (1.8%)	1,045 (98.2%)	—	—				
Bats roosting in the home, close to the home or at the place of work					>0.9			
Yes	5 (1.7%)	288 (98.3%)	0.95	0.31, 2.52				
No	14 (1.8%)	770 (98.2%)	—	—				
Age [median (IQR)]	33 (27, 40)	30 (23, 42)	1.00	0.96, 1.04	>0.9			
Age					0.6			
18 to 27	6 (1.3%)	444 (98.7%)	—	—				
28 to 37	7 (2.4%)	286 (97.6%)	1.81	0.60, 5.68				
38 to 47	4 (2.1%)	184 (97.9%)	1.61	0.41, 5.70				
48 to 57	2 (1.6%)	120 (98.4%)	1.23	0.18, 5.43				
58 to 77	0 (0.0%)	43 (100.0%)	0.00					
Housing condition					0.2			
Permanent	8 (1.4%)	577 (98.6%)	0.51	0.19, 1.34				
Semi-permanent	9 (2.7%)	329 (97.3%)	—	—				
Temporary	1 (0.6%)	162 (99.4%)	0.23	0.01, 1.22				
Travelled away from home in the past three weeks					0.3			
Yes	1 (0.8%)	131 (99.2%)	0.40	0.02, 1.96				
No	18 (1.9%)	942 (98.1%)	—	—				
Care for domestic animals					0.8			
Yes	10 (1.7%)	592 (98.3%)	0.91	0.36, 2.31				
No	9 (1.8%)	485 (98.2%)	—	—				
Tick bite in the past 3 weeks					0.6			
Yes	0 (0.0%)	9 (100.0%)	0.00					
No	19 (1.7%)	1,068 (98.3%)	—	—				
Rodents or evidence of rodents in the house					**0.039**			**0.041**
Yes	17 (2.2%)	746 (97.8%)	3.73	1.06, 23.6		3.73	1.05, 23.8	
No	2 (0.6%)	327 (99.4%)	—	—		—	—	

[1] n (%); Median (IQR)

[2] OR = Odds Ratio

[3] AOR = Adjusted Odds Ratio

[4] removed from multivariable regression due to 154 'Don't know' values

Table A.8: Exploratory analysis of all variables for possible risk of RVFV seropositivity (HCW study Chapter 2).

````
---
title: "Maps"
author: "Marina"
date: '2022-08-01'
output:
  pdf_document: default
  html_document: default
---

```{r setup, include=FALSE}
knitr::opts_chunk$set(echo = F, warning = F, message = F)
```


```{r libraries}

library(tidyverse)
library(readxl)
library(sf)
library(ggrepel)
library(terra)
library(raster) #problem it is soon outdated, but raster function still works
library(devtools)
#devtools::install_github('wpgp/wopr')
#library(wopr)

```

```{r shape Uganda}
#downloaded shapefiles from this webpage 14.June 2022
#https://data.unhcr.org/en/documents/details/83043


#shapefile for Uganda
shape_Ug <- st_read("data/Uganda_Districts-2020---136-wgs84/Uganda_Districts-2020---136-
wgs84.shp")
#row.names(shape_Ug) <- shape_Ug$d #make it easier for later, so now the IDs are the district
names


```



```{r forest cover raster data - Hansen - excluded}
#way to heavy - use copernicus instead

#get forest cover raster data from
#https://earthenginepartners.appspot.com/science-2013-global-forest/download_v1.7.html
#citation;
#IMPORTANT this is from the year 2000 - James got something from 2010????????
#Hansen, M.C., Potapov, P.V., Moore, R., Hancher, M., Turubanova, S.A., Tyukavina, A., Thau, D.,
Stehman, S.V., Goetz, S.J., Loveland, T.R., Kommareddy, A., Egorov, A., Chini, L., Justice,
C.O., and Townshend, J.R.G., 2013, High-Resolution Global Maps of 21st-Century Forest Cover
Change: Science, v. 342, no. 6160, p. 850-853, at
#http://www.sciencemag.org/content/342/6160/850.abstract
#10.1126/science.1244693
#
# forest1 <- rast("Geospatial data/Treecover data/Hansen_GFC-2019-
v1.7_treecover2000_00N_020E.tif")
# forest2 <- rast("Geospatial data/Treecover data/Hansen_GFC-2019-
v1.7_treecover2000_00N_030E.tif")
# forest3 <- rast("Geospatial data/Treecover data/Hansen_GFC-2019-
v1.7_treecover2000_10N_020E.tif")
# forest4 <- rast("Geospatial data/Treecover data/Hansen_GFC-2019-
v1.7_treecover2000_10N_030E.tif")
#
# forest <- terra::mosaic(forest1,forest2,forest3,forest4,fun="mean")
# UGA_forest <- terra::crop(forest,extent(shape_Ug))
#
# #get areas to mask (permanent water bodies) from global forest dataset
# #can this be used for others too?
# #changer raster() to rast() - now use terra library
# mask <- rast("Geospatial data/Treecover data/Hansen_GFC-2019-v1.7_datamask_00N_020E.tif")
# sources(mask)
````
````

```
# hasValues(mask)
# plot(mask)
# mask1 <- rast("Geospatial data/Treecover data/Hansen_GFC-2019-v1.7_datamask_00N_030E.tif")
# mask2 <- rast("Geospatial data/Treecover data/Hansen_GFC-2019-v1.7_datamask_10N_020E.tif")
# mask3 <- rast("Geospatial data/Treecover data/Hansen_GFC-2019-v1.7_datamask_10N_030E.tif")
# maskm <- merge(mask,mask1,mask2,mask3)
# maskm <- terra::mosaic(mask,mask1,mask2,mask3,fun="mean")
# ?mosaic
# plot(maskm)
# mask_UGA <- terra::crop(maskm,extent(shape_Ug))
# ?crop
# plot(mask_UGA)
#
# #mask areas of forest with water
# UGA_forest.masked <- terra::mask(UGA_forest, mask_UGA,maskvalues=2, updatevalue=NA)
# ?mask
# plot(UGA_forest.masked)
#
# rm(forest1)
# rm(forest2)
# rm(forest3)
# rm(forest4)
# rm(forest)
# rm(UGA_forest)
#
# #runs for more than an hour and doesnt work
# #forest_cover <- terra::extract(UGA_forest.masked,vect(shape_Ug), fun="mean",na.rm=TRUE)
# district_1 <- shape_Ug[1,]
# forest_cover <- terra::extract(UGA_forest.masked,vect(district_1), fun="mean", exact=F,
na.rm=TRUE)
# ?extract
```


```{r land surface temperature}

#https://neo.gsfc.nasa.gov/view.php?datasetId=MOD_LSTD_M
#June 2022
#NASA earth observations
#1.0 degrees 360x180

#mask - all water bodies for NA
land_surface_temp.mask <- rast("data/MOD_LSTD_M_2022-06-01_rgb_3600x1800.FLOAT.TIFF")
land_surface_temp.mask <- terra::crop(land_surface_temp.mask,extent(shape_Ug))
plot(land_surface_temp.mask)

land_surface_temp <- rast("data/MOD_LSTD_M_2022-06-01_rgb_3600x1800.TIFF")
land_surface_temp <- terra::crop(land_surface_temp,extent(shape_Ug))
plot(land_surface_temp)
#I dont understand why the numbers are so high! above 100
UGA_land_surface_temp.masked <- terra::mask(land_surface_temp,
land_surface_temp.mask,maskvalues=99999, updatevalue=NA)
plot(UGA_land_surface_temp.masked)
terra::extract(UGA_land_surface_temp.masked,vect(shape_Ug[6,]),fun="mean",na.rm=TRUE)
land_surface_temp.mean <-  terra::extract(UGA_land_surface_temp.masked,vect(shape_Ug),
                                          fun="mean",na.rm=TRUE)

#still numbers are high, but i cant figure out why
land_surface_temp.mean



```



```{r worldclim }
#worldclim bio dataset - Annual precipitation
## get worldclim bio raster tiles that cover all of Uganda (need to combine 4 as Uganda
straddles all 4)
# bio <- raster::getData(name = 'worldclim',var='bio',res=0.5,lon=c(32.290275),lat=c(0.347596))
# bio1 <- raster::getData(name = 'worldclim',var='bio',res=0.5,lon=31,lat=-1)
# bio2 <- raster::getData(name = 'worldclim',var='bio',res=0.5,lon=c(29), lat=c(0))
# bio3 <- raster::getData(name = 'worldclim',var='bio',res=0.5,lon=c(29), lat=c(1))
```

```
# ## combine bio raster tiles
# bio_combined <- mosaic(bio, bio1,bio2, bio3,fun=mean)
# ## crop bio raster tiles to Uganda area
# bio_UGA <- raster::crop(bio_combined,extent(shape_Ug))
# ## remove redundant objects
# rm(bio)
# rm(bio1)
# rm(bio2)
# rm(bio3)
# rm(bio_combined)
# #no need to mask data, James checked
# bionames <- c("Annual_mean_temp","Mean_diurnal_range",
#               "Isothermality","Temp_seasonality",
#               "Max_temp_warmest_month","Min_temp_coldest_month",
#               "Temp_annual_range",
#               "Mean_temp_wettest_quarter",
#               "Mean_temp_driest_quarter",
#               "Mean_temp_warmest_quarter",
#               "Mean_temp_coldest_quarter","Annual_precipitation",
#               "Precipitation_wettest_month",
#               "Precipitation_driest_month","Precipitation_seasonality (Coefficient of
Variation)",
#               "Precipitation_wettest_quarter",
#               "Precipitation_driest_quarter","Precipitation_warmest_quarter",
#               "Precipitation_coldest_quarter")
# names(bio_UGA) <- bionames

#worldclim data is 20 years mean
#not very precice, only interesting for strong differences

mean_temp_warmest_quarter <-rast("data/wc2.1_30s_bio_10.tif")
mean_temp_warmest_quarter <-terra::crop(mean_temp_warmest_quarter,extent(shape_Ug))
mean_temp_warmest_quarter.mean <-
terra::extract(mean_temp_warmest_quarter,vect(shape_Ug),fun="mean",na.rm=TRUE)

mean_temp_coldest_quarter <-rast("data/wc2.1_30s_bio_11.tif")
mean_temp_coldest_quarter <-terra::crop(mean_temp_coldest_quarter,extent(shape_Ug))
mean_temp_coldest_quarter.mean <-
terra::extract(mean_temp_coldest_quarter,vect(shape_Ug),fun="mean",na.rm=TRUE)

annual_precipitation <-rast("data/wc2.1_30s_bio_12.tif")
annual_precipitation <-terra::crop(annual_precipitation,extent(shape_Ug))
annual_precipitation.mean <-
terra::extract(annual_precipitation,vect(shape_Ug),fun="mean",na.rm=TRUE)

isothermality <-rast("data/wc2.1_30s_bio_3.tif")
isothermality <-terra::crop(isothermality,extent(shape_Ug))
isothermality.mean <- terra::extract(isothermality,vect(shape_Ug),fun="mean",na.rm=TRUE)



```


```{r livestock density}
## Get livestock density data
## potentiall newer dataset http://www.fao.org/ag/againfo/resources/en/glw/glw_dens.html - USE
THIS DATASET
# https://www.fao.org/livestock-systems/global-distributions/en/ ##################
## Food Agricultral Organisation global livestock dataset
## animals per square km
#  spatial resolution of 5 minutes of arc,  approximately 10 km at the equator
# dataset 2010

#cows
#https://www.fao.org/livestock-systems/global-distributions/cattle/en/
#https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/GIVQ75
#cows<-rast("Geospatial data/5_Ct_2010_Da.tif")
cows<-rast("data/6_Ct_2015_Aw.tif") #i dont see a difference, but this says areal-weighted
#cows<-rast("Geospatial data/7_Ct_2010_Ps.tif") #thats the 0 or 1
cows<-terra::crop(cows,extent(shape_Ug))
cows.mean <- terra::extract(cows,vect(shape_Ug),fun="mean",na.rm=TRUE)

#pigs
pigs<-rast("data/6_Pg_2015_Aw.tif")
pigs<-terra::crop(pigs,extent(shape_Ug))
```

```
pigs.mean <- terra::extract(pigs,vect(shape_Ug),fun="mean",na.rm=TRUE)

#sheep
sheep<-rast("data/6_Sh_2015_Aw.tif")
sheep<-terra::crop(sheep,extent(shape_Ug))
sheep.mean <- terra::extract(sheep,vect(shape_Ug),fun="mean",na.rm=TRUE)
names(sheep.mean)

#goat
goat<-rast("data/6_Gt_2015_Aw.tif")
goat<-terra::crop(goat,extent(shape_Ug))
goat.mean <- terra::extract(goat,vect(shape_Ug),fun="mean",na.rm=TRUE)

#chicken
chicken<-rast("data/6_Ch_2015_Aw.tif")
chicken<-terra::crop(chicken,extent(shape_Ug))
chicken.mean <- terra::extract(chicken,vect(shape_Ug),fun="mean",na.rm=TRUE)



## combine them together?
#livestock<-cows+pigs+sheep+goats
#livestock.mean <- terra::extract(livestock,vect(shape_Ug),fun="mean",na.rm=TRUE)


```



```{r land coverage}

#https://zenodo.org/record/5848610#.YukF0S8w30o
#Copernicus Global Land Service: Global biome cluster layer for the 100m global land cover
processing line
#Marcel Buchhorn
# land_coverage_shape <- st_read("Geospatial
data/biome_cluster_shapefile/ProbaV_UTM_LC100_biome_clusters_V3_global.shp")
# ggplot(land_coverage_shape) +
#   geom_sf()
#from 2015

#Crops coverage
#https://zenodo.org/record/3518056#.Yup35y8w30o
#Copernicus
crops_coverage <- rast("data/ProbaV_LC100_epoch2015-base_Africa_v2.1.1_crops-coverfraction-
layer_EPSG-4326.tif")
crops_coverage <- terra::crop(crops_coverage,extent(shape_Ug))
crops_coverage.mean <-  terra::extract(crops_coverage,vect(shape_Ug),
                                       fun="mean",na.rm=TRUE)

#Urban cover
#https://zenodo.org/record/3518056#.Yup35y8w30o
#Copernicus
urban_coverage <- rast("data/ProbaV_LC100_epoch2015-base_Africa_v2.1.1_urban-coverfraction-
layer_EPSG-4326.tif")
urban_coverage <- terra::crop(urban_coverage,extent(shape_Ug))
urban_coverage.mean <-  terra::extract(urban_coverage,vect(shape_Ug),
                                       fun="mean",na.rm=TRUE)


#Tree cover
#https://zenodo.org/record/3518056#.Yup35y8w30o
#Copernicus
tree_coverage <- rast("data/ProbaV_LC100_epoch2015-base_Africa_v2.1.1_tree-coverfraction-
layer_EPSG-4326.tif")
tree_coverage <- terra::crop(tree_coverage,extent(shape_Ug))
tree_coverage.mean <-  terra::extract(tree_coverage,vect(shape_Ug),
                                       fun="mean",na.rm=TRUE)



```


```{r elevation}
```

```
#elevation data
#would be great if we find another source

elevation <- raster::getData('alt',country="UGA",mask=TRUE) #old use of raster!!!! but cant find
a better dataset online
elevation <- rast(elevation) #change into SpatRaster object
#elevation <- rast(elevation * 1) #need to add the 1 somewhen it only worked like that
elevation.mean <- terra::extract(elevation,vect(shape_Ug),fun="mean",na.rm=TRUE)


```

```{r population density}

#population density
#26th July 2022  https://hub.worldpop.org/geodata/summary?id=49328

#population_density <- read_csv("Geospatial data/uga_pd_2020_1km_UNadj_ASCII_XYZ.csv")
population_density <- rast("data/uga_pd_2020_1km_UNadj.tif") #new from terra
#population_density_tif <- raster("Geospatial data/uga_pd_2020_1km_UNadj.tif") #old from raster
population_density.log <- log(population_density)
population_density.log.mean <-  terra::extract(population_density.log,vect(shape_Ug),
                                               fun="mean",na.rm=TRUE)
#row.names(population_density.log.mean) <- shape_Ug$d
#population_density_log_mean["Kampala",]
#population_density.log.mean - contains the mean population in this district - can be used



```



```{r combine datasets}

#all information collected to this point about districts
data_variables <- read_csv("data/districts_variables.csv")
row.names(data_variables)
names(data_variables)
#exlude the city district, because we dont have them as a shape file
data_m_variables <- data_variables[which(data_variables$City == 0),]




#combine data and summarize in district_variables

data_m_variables

#population
data_m_variables$population_density.log.mean <-
population_density.log.mean$uga_pd_2020_1km_UNadj
#elevation
data_m_variables$elevation.mean <- elevation.mean$UGA_msk_alt
#tree coverage
data_m_variables$tree_coverage.mean <-
tree_coverage.mean$ProbaV_LC100_epoch2015.base_Africa_v2.1.1_tree.coverfraction.layer_EPSG.4326
#urban coverage
data_m_variables$urban_coverage.mean <-
urban_coverage.mean$ProbaV_LC100_epoch2015.base_Africa_v2.1.1_urban.coverfraction.layer_EPSG.4326
#crop coverage
data_m_variables$crops_coverage.mean <-
crops_coverage.mean$ProbaV_LC100_epoch2015.base_Africa_v2.1.1_crops.coverfraction.layer_EPSG.4326
#land surface temperatur
data_m_variables$land_surface_temp.mean <-
land_surface_temp.mean$MOD_LSTD_M_2022.06.01_rgb_3600x1800
#cows
data_m_variables$cows.mean <- cows.mean$X6_Ct_2015_Aw
#pigs
data_m_variables$pigs.mean <- pigs.mean$X6_Pg_2015_Aw
#sheep
data_m_variables$sheep.mean <- sheep.mean$X6_Sh_2015_Aw
#goat
data_m_variables$goat.mean <- goat.mean$X6_Gt_2015_Aw
#chicken
data_m_variables$chicken.mean <- chicken.mean$X6_Ch_2015_Aw
```

```
#annual precipitation
data_m_variables$annual_precipitation.mean <- annual_precipitation.mean$wc2.1_30s_bio_12
#max temp
data_m_variables$mean_temp_warmest_quarter.mean <-
mean_temp_warmest_quarter.mean$wc2.1_30s_bio_10
#min temp
data_m_variables$mean_temp_coldest_quarter.mean <-
mean_temp_coldest_quarter.mean$wc2.1_30s_bio_11
#isothermality
data_m_variables$isothermality.mean <- isothermality.mean$wc2.1_30s_bio_3



#check for the class of the variables
names(data_m_variables)
class(data_m_variables$cattle_corridor)
data_m_variables$cattle_corridor <- as.factor(data_m_variables$cattle_corridor)
class(data_m_variables$outbreak)
data_m_variables$outbreak <- as.factor(data_m_variables$outbreak)



#write
write.csv(data_m_variables, file = "data/districts_variable_m_addon.csv")


```
```
```

```
library(tidyverse)  # data manipulation, read_csv
library(cluster)    # clustering algorithms
library(factoextra) # clustering algorithms & visualization
library(gridExtra) #for plotting more than one graph togehter
library(hkclustering) #for ensemble clustering for kmeans analysis
library(clustMixType) #for kproto function



#read in the variables which i created in the excel sheet and in env_01
df2 <- read_csv("District_selection/data/District_selection_variables_Uganda.csv")

names(df2)
head(df2)

class(df2$district) #stays character
#df2$province <- as.factor(df2$province)
#df2$cattle_corridor <- as.factor(df2$cattle_corridor)
#df2$border_crossing <- as.factor(df2$border_crossing)
df2$national_park <- as.factor(df2$national_park)

df2 <- df2[,c(-1)] #remove extracolumns which i dont need
#be careful not to delete wrong things


names(df2)
row.names(df2)
table(df2$district)
row.names(df2) <- df2$district
df2 <- df2[,-1]
head(df2)

#Kmeans clustering
#first make the normal run and find out the number of k i want
#make all the plots for one example
#then use the ensemble clustering to find out the best
#hkclustering(df, numbk, t)
#results.hkclust <- hkclustering(cluster_first, 2, 20)
#results.hkclust<-hkclustering(df,4,100)
#centroidssummary(results.hkclust)
#with(results.hkclust, pairs(results.hkclust[,1:2], col=c(1:10)[results.hkclust[,3]]))


#K-prototypes####
names(df2)
#make sure that all categorical variables are factor


#scale all mean variables
df2_scale <- df2
df2_scale[,c(1:14)] <- scale(df2[,c(1:14)])

names(df2_scale)
dim(df2_scale)
dim(df2)


#how many centers do i need?
Es <- numeric(30)
for(i in 1:30){
  kpres <- kproto(df2_scale, k = i)
  Es[i] <- kpres$tot.withinss
}
plot(1:30, Es, type = "b", ylab = "Total Within Sum Of Squares", xlab = "Number of clusters")
#based on elbow method, i think 12 is a good number
pdf("District_selection/output/Total Within Sum Of Squares_Uganda.pdf")
plot(1:30, Es, type = "b", ylab = "Total Within Sum Of Squares", xlab = "Number of clusters")
dev.off()


kpres <- validation_kproto(method = "silhouette", data=df2_scale, k = c(2:16))
kpres$index_opt
#indicates that it should be 2 - not ideal obviously, maybe says only Uganda Kenya?



#total withiness - difference between the samoples
total_withinss <- c()
for(i in 1:25) {
  kproto <- kproto(df2_scale,
                   k = i,
                   nstart = 100)
  total_withinss[i] <- kproto$tot.withinss
```

```
}
tibble(k = 1:length(total_withinss),
       total_error = total_withinss) %>%
  ggplot(aes(x = k,
             y = total_error)) +
  geom_point(size = 2) +
  geom_line() +
  theme_bw() +
  labs( x = "number of clusters", y = "tot.withinss") +
  geom_text(x = 3, y = total_withinss[3],
            label = "ELBOW", alpha = 0.5,
            color = "blue", size = 5)



#make sure that i can repeat the caluclations multiple time
#i set the seed to a complete random
#and then save it

#random_seed_kproto <- .Random.seed
#dont change that anymore
#it is set and should be used like that
#write(random_seed_kproto,"data/random_seed_kproto.csv")
random_seed_kproto <- read_csv("District_selection/data/random_seed_kproto_k.csv")
random_seed_kproto$x
set.seed(random_seed_kproto$x)


#so i use 12 centers
Uga_kproto <- kproto(df2_scale, k = 12, nstart = 100)
#nstart is how often it is repeated with random
#initializations and the best will be taken out
#- the higher the more often it randomly starts somewhere and then picks the best at the end
#k is the number of centers
?kproto
Uga_kproto$centers
dim(data.frame(Uga_kproto$cluster))



#visualisation of centers and variables####
#not really needed
getAnywhere(clprofiles) #shows the function behind
as.data.frame(df2_scale) #needed to change that

summary(KenUga_kproto) #gives all information


getPalette = colorRampPalette(brewer.pal(4, "Set1"))
my.clprofiles <- function (object, x, vars = NULL, col = NULL)
{
  library(RColorBrewer)
  if (length(object$cluster) != nrow(x))
    stop("Size of x does not match cluster result!")
  if (is.null(vars))
    vars <- 1:ncol(x)
  if (!is.numeric(vars))
    vars <- sapply(vars, function(z) return(which(colnames(x) ==
                                                    z)))
  if (length(vars) < 1)
    stop("Specified variable names do not match x!")
  if (is.null(col)) {
    k <- max(unique(object$cluster))
    if (k > 2)
      col <- getPalette(k)
    if (k == 2)
      col <- c("lightblue", "orange")
    if (k == 1)
      col <- "lightblue"
  }
  clusids <- sort(unique(object$cluster))
  if (length(col) != max(clusids))
    warning("Length of col should match number of clusters!")
  #remove prompt
  #par(ask = TRUE)
  for (i in vars) {
    if (is.numeric(x[, i])) {
      boxplot(x[, i] ~ object$cluster, col = col, main = colnames(x)[i])
      legend("topright", legend = clusids, fill = col)
    }
    if (is.factor(x[, i])) {
      tab <- table(x[, i], object$cluster)
      for (j in 1:length(object$size)) tab[, j] <- tab[,
```

```
                                                        j]/object$size[j]
      barplot(t(tab), beside = TRUE, main = colnames(x)[i],
              col = col)
    }
  }
  #par(ask = FALSE)
  invisible()
}

pdf("District_selection/output/kproto_cluster.pdf")
my.clprofiles(KenUga_kproto,as.data.frame(df2_scale))
dev.off()



#add to dataset for furter work####
names(Uga_kproto)
Uga_kproto$cluster
Uga_kproto$centers
df3 <- read_csv("District_selection/data/District_selection_variables_Uganda.csv")
df3$kproto12_centers <- Uga_kproto$cluster

write.csv(df3, file = "District_selection/data/District_selection_variables_Uganda_cluster12.csv")



#remove centers for analysis
write.csv(Uga_kproto$centers, file =
"District_selection/output/District_selection_variables_Uganda_centers.csv")


#make a graph
library(sf) #for st_read
library(tidyverse) #for full_join


shape_Ug <- st_read("District_selection/data/Uganda_Districts-2020---136-wgs84/Uganda_Districts-2020--
-136-wgs84.shp")

names(shape_Ug)[2] <- "district"



shape_Ug_add <- full_join(shape_Ug,df3,by="district")




#check for the class of the variables
names(shape_Ug_add)
#check for class if needed
class(shape_Ug_add$kproto12_centers)
shape_Ug_add$kproto12_centers <- as.factor(shape_Ug_add$kproto12_centers)


#need 13 colours - i can choose any i want
farbvector <- c('#e6194b', '#3cb44b', '#ffe119', '#4363d8', '#f58231', '#911eb4', '#46f0f0', '#f032e6',
'#bcf60c', '#fabebe', '#008080', '#e6beff',"grey")

pdf("District_selection/output/Uganda_Kprototype analysis together_12centers.pdf")
ggplot(shape_Ug_add) +
  geom_sf(aes(fill = kproto12_centers), lwd = 0.1) +
  labs(title = "K Prototype clustering with 13 centers",
       caption = "") +
  scale_fill_manual(values= farbvector)
dev.off()
```

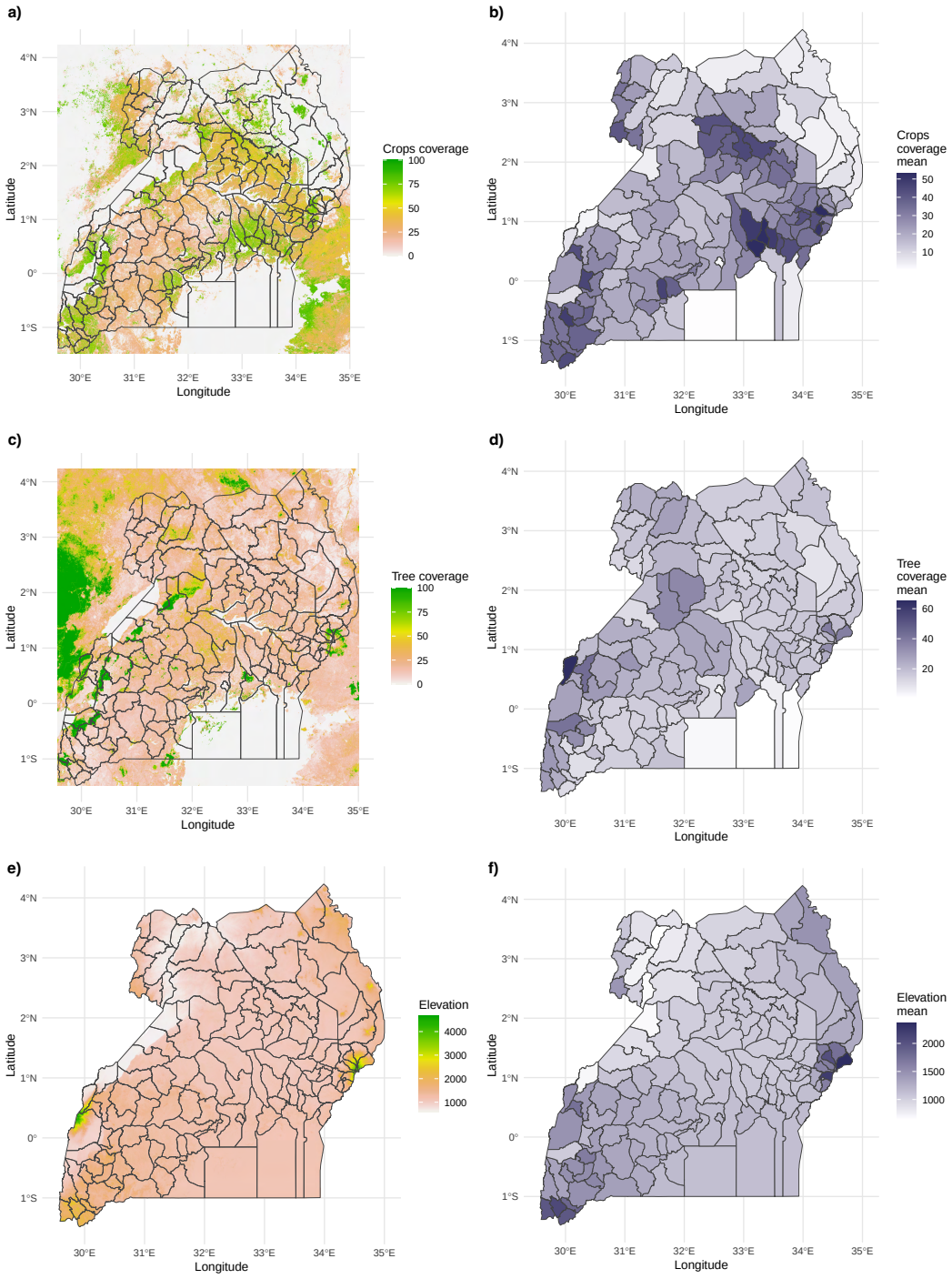

Figure A.1: **Further environmental variables** used for the K-prototype analysis. All maps represent Uganda and the 136 districts. Presented are coverages from Buchhorn *et al.* 2019. **(a)** Crops coverage. **(b)** Crops coverage as the mean per district values used for the K-prototype analysis. **(c)** Tree coverage. **(d)** Tree coverage as the mean per district. **(e)** Elevation within Uganda. **(f)** Elevation as the mean per district. As part of Chapter 3.

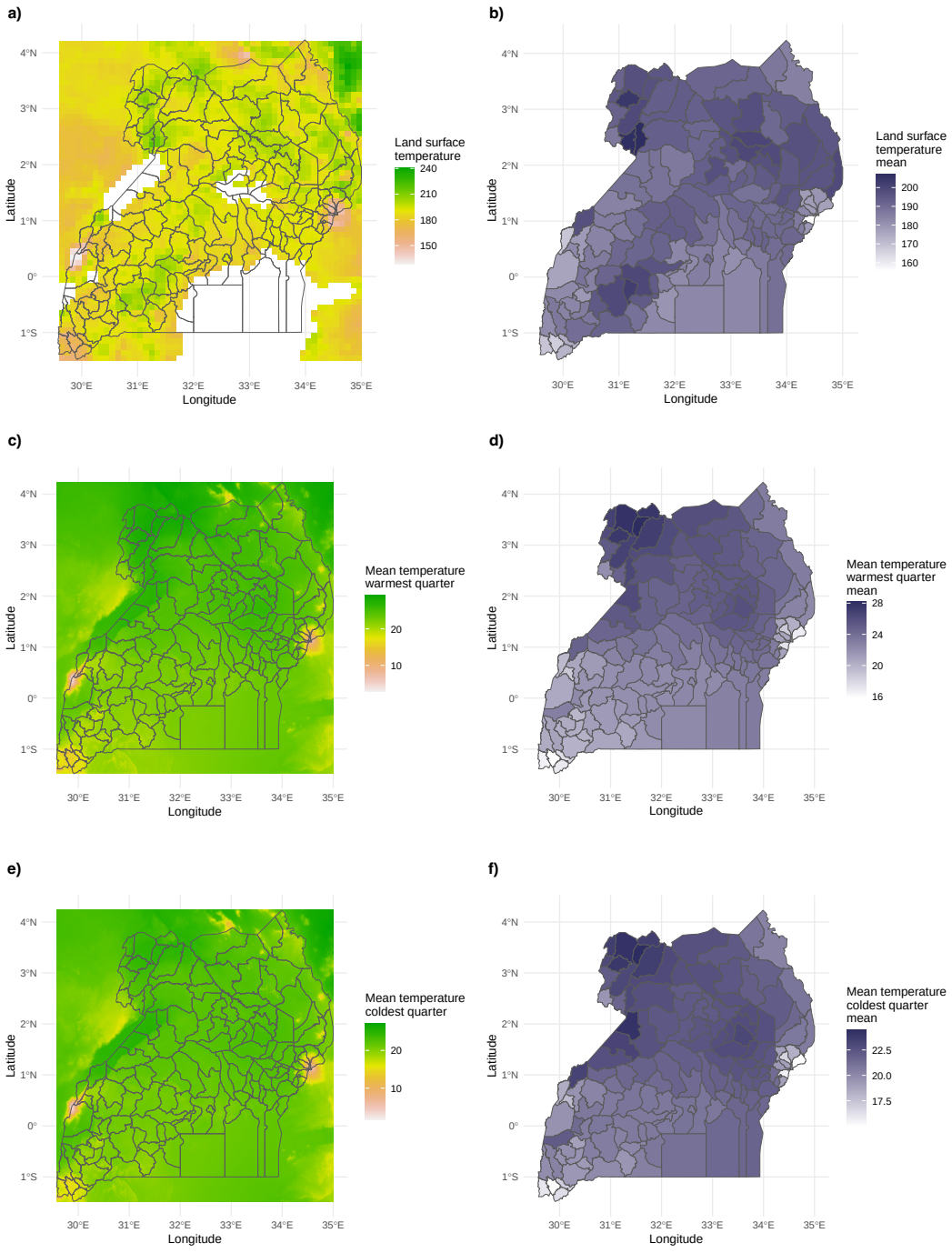

Figure A.2: **Further environmental variables** used for the K-prototype analysis. All maps represent Uganda and the 136 districts. **(a)** Land surface temperatures downloaded for June 2022 from NEO (Nasa Earth Observations), 2022. **(b)** Land surface as the mean per district values used for the K-prototype analysis. **(c)** Mean temperature of the warmest quarter. **(d)** Mean temperature of the warmest quarter as the mean per district. **(e)** Mean temperature of the coldest quarter. **(f)** Mean temperature of the coldest quarter as the mean per district. As part of Chapter 3.

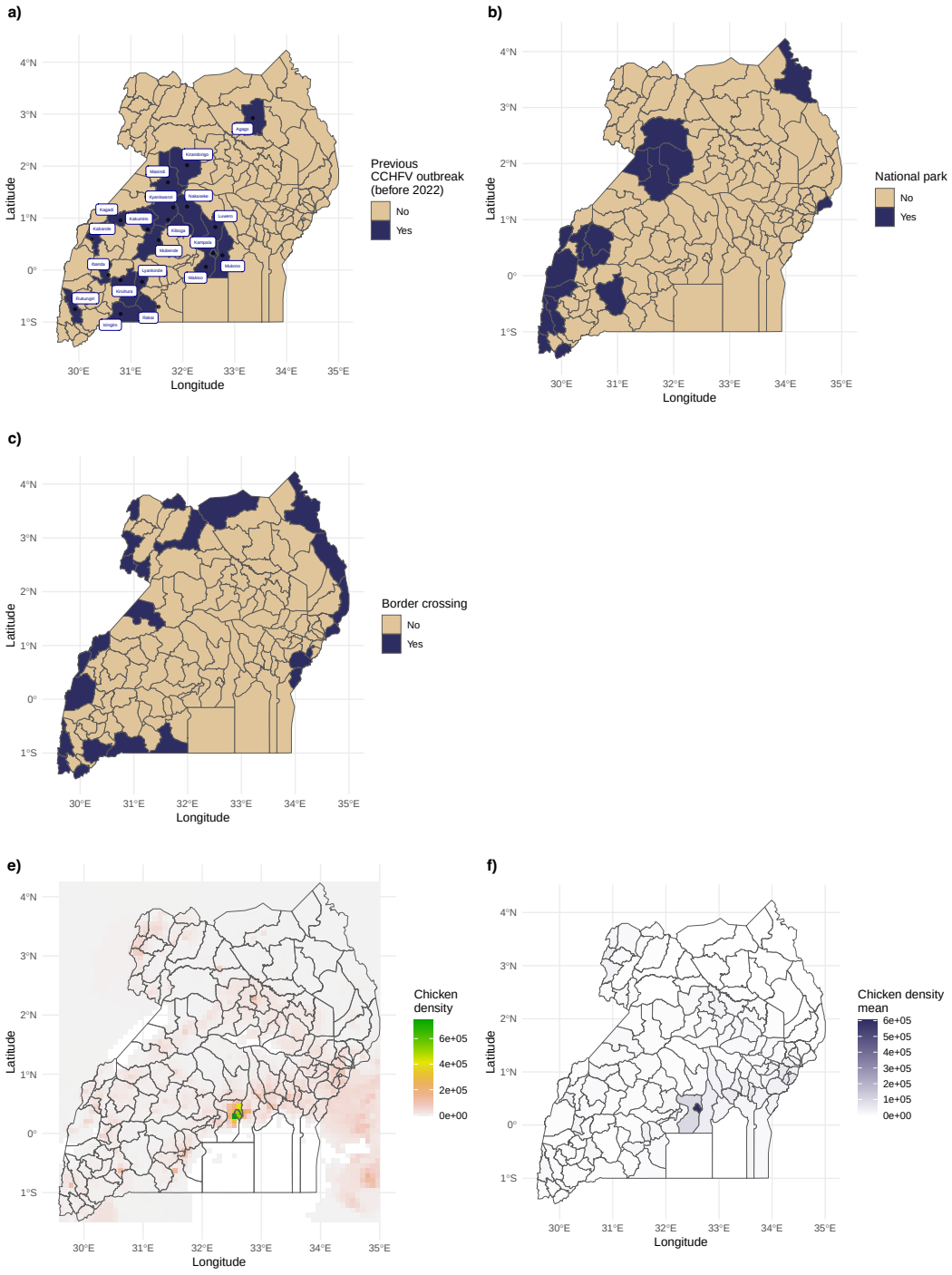

Figure A.3: **Further socioecological variables** used for the K-prototype analysis. All maps represent Uganda and the 136 districts. **(a)** Previous CCHFV outbreaks in Uganda till 2022. **(b)** Districts with national parks within their borders. **(c)** Districts with large border crossing points into neighbouring countries. **(e)** Chicken density from FAO, 2015. **(f)** Chicken density as the mean per district for K-prototype analysis. As part of Chapter 3.

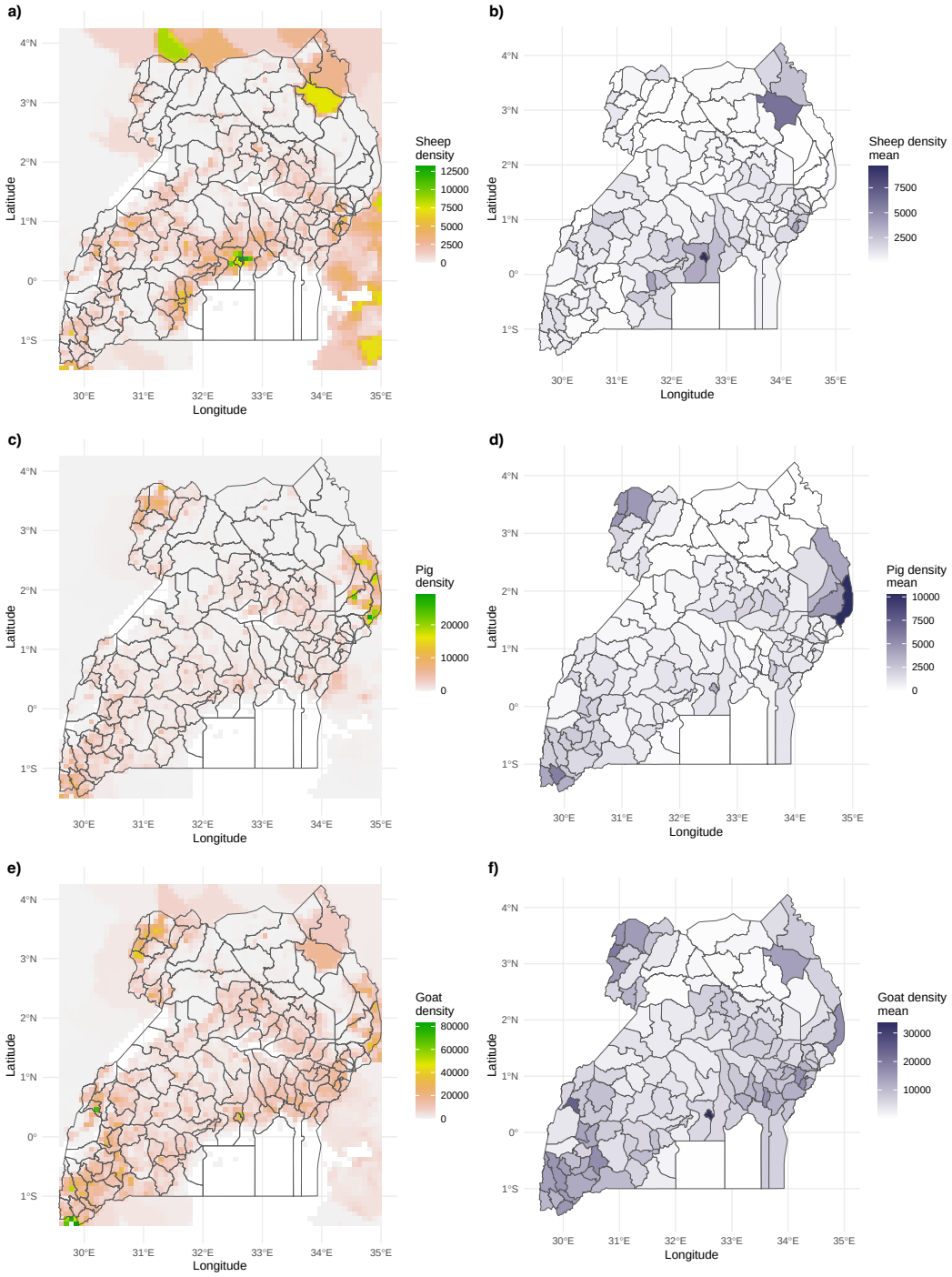

Figure A.4: **Further socioecological variables** used for the K-prototype analysis. All maps represent Uganda and the 136 districts. All animal densities are downloaded from FAO, 2015. **(a)** Sheep density. **(b)** Sheep density as the mean per district. **(c)** Pig density. **(d)** Pig density as the mean per district. **(e)** Goat density. **(f)** Goat density as the mean per district. As part of Chapter 3.

```
#read the files from Nvivo
#create a big excel document which contains the codes as rows and the files as columns


# Load necessary libraries
library(dplyr)
library(writexl) #to write an excel file
library(openxlsx)


# Define the directory containing my text files
directory_path <- "All codes"


# Get a list of all txt files in the directory
file_list <- list.files(directory_path, pattern = "\\.txt$", full.names = TRUE)

# Initialize an empty list to store data from each file
data_list <- list()

# Loop over each file to read and process
for (file in file_list) {
  # Read the content of the file
  file_content <- readLines(file, encoding = "UTF-8", warn = TRUE)
  # Remove blank lines (lines that are empty or contain only whitespace)
  file_content <- file_content[!grepl("^\\s*$", file_content)]

  # Extract the filename without extension to use as row name
  row_name <- tools::file_path_sans_ext(basename(file))

  # Find lines that start with "Files\\" and extract content between "Files\\" and "-"
  sections <- grep("^Files\\\\", file_content)

  # Create a named vector to store sections
  file_data <- c()

  # Loop over each found section
  for (i in seq_along(sections)) {
    # Extract the section name for the column header
    header <- sub("Files\\\\(.*?) -.*", "\\1", file_content[sections[i]])

    # Determine the start and end of the content for this section
    start <- sections[i] + 1
    end <- if (i < length(sections)) sections[i + 1] - 1 else length(file_content)

    # Extract the relevant lines
    content <- file_content[start:end]

    # Add a new line before lines starting with "Reference"
    content <- gsub("^(Reference)", "\n\\1", content)  # Add four spaces before
"Reference"

    # Preserve the paragraph and line breaks by collapsing with newline characters
    content <- paste(content, collapse = "\n")

    # Store in the vector with header as name
    file_data[header] <- content
  }

  # Add the file's data to the list using the row name
  data_list[[row_name]] <- file_data
}

# Combine all the individual file data into a data frame
data_df <- bind_rows(data_list, .id = "File")


# Display the resulting data frame
print(data_df)
```

```
write_xlsx(data_df, path = "All codes/00_all_codes_combined_unsorted.xlsx")



# Sort the rows, so they are in the same order as in the codebook
data_df$File
custom_order <- c(
  "01_Knowledge and awareness of haemorrhagic diseases",
  "CCHF knowledge",
  "Ebola knowledge",
  "Other knowledge",
  "02_Knowledge of ticks",
  "Dangers of ticks",
  "Dangers of ticks to specifically humans",
  "General awareness",
  "03_Community cases CCHHF",
  "CCHF diagnoses and treatment",
  "CCHF symptoms",
  "CCHF transmission",
  "Community cases CCHFV general",
  "04_Health seeking behaviour",
  "05_Changes during and after sickness of CCHF",
  "06_Tick bites",
  "Most affected by tick bites",
  "In animals = most affected",
  "In humans - most affected",
  "Removal and discarding of ticks",
  "Animals and unspecified - removal",
  "Humans (specified) - removal",
  "Symptoms and treatment of tick bites",
  "Animals and unspecified - Symptoms and treatment",
  "Humans (specified) - Symptoms and treatment",
  "Tick bites community cases",
  "Animals and unspecified - tick bites",
  "Humans (specified) - tick bites",
  "07_possible risks for tick bites",
  "Animals",
  "Activities with animals",
  "Animal keeping",
  "Animal tick control",
  "Cultural practices",
  "People handling animals",
  "Environment factors",
  "District-wide environment",
  "Environment tick control",
  "Living environment",
  "Presence of ticks",
  "Rodents",
  "Wild animals",
  "08_Possible risks for direct transmission",
  "Cultural and religious practices",
  "Caring for sick or deceased person",
  "Handling animal products",
  "Eating and selling animal product",
  "Blood",
  "Meat",
  "Milk",
  "Eating ticks",
  "Slaugthering",
  "Location",
  "Method",
  "People",
  "PPE",
  "Treatment and diagnosis of animals",
  "09_Other",
  "District procedure for suspected CCHF case",
  "Hopsital procedure for suspected CCHF case",
```

```
  "10_Recommendations and ideas",
  "Adherence to recommendations",
  "Feedback",
  "Research",
  "Sensitisation and awareness",
  "Supply and focus by the government",
  "11_Unknown code"
)

# Ensure the File column is a factor with levels in the desired order
data_df$File <- factor(data_df$File, levels = custom_order)
codes <- as.data.frame(custom_order)
names(codes) <- "File"
merge_df <- merge(data_df,codes,by="File",all = TRUE)
merge_df$File




# Define custom styles
# Create a workbook and add a worksheet
wb <- createWorkbook()
addWorksheet(wb, "Sheet1")


# Write data to the worksheet
writeData(wb, sheet = "Sheet1", x = merge_df, colNames = TRUE, rowNames = FALSE)


# Apply styles
headerStyle <- createStyle(
  fontSize = 12,
  halign = "center",
  valign = "center",
  border = "TopBottomLeftRight"
)

cellStyle <- createStyle(
  fontSize = 10,
  wrapText = TRUE,
  valign = "top"
)
addStyle(wb, sheet = "Sheet1", style = headerStyle, rows = 1, cols = 1:ncol(merge_df),
gridExpand = TRUE)
addStyle(wb, sheet = "Sheet1", style = cellStyle, rows = 2:(nrow(merge_df) + 1), cols =
1:ncol(merge_df), gridExpand = TRUE)
setColWidths(wb, sheet = "Sheet1", cols = 1:ncol(merge_df), widths = 60)

# Save the workbook
saveWorkbook(wb, file = "All codes/00_all_codes_combined_formated.xlsx", overwrite =
TRUE)
```

Figure A.5: **Example 1 Commonalities art exhibition.** As part of Chapter 6.

Lily at Sipi Falls, Uganda - 25th February 2024

Magnolia flowers in London, UK - 26th March 2023

Figure A.6: **Example 2 Commonalities art exhibition.** As part of Chapter 6.

Appendix B

Ethical approval and others

UNHRO
Uganda National Health Research Organisation

Uganda Virus Research Institute
Plot 51-59, Nakiwogo Road, Entebbe
P.O. Box 49, Entebbe-Uganda
Tel: +256 414 320 385 / 6
Fax: +256 414 320 483
Email: directoruvri@uvri.go.ug

REPUBLIC OF UGANDA

Our Ref: GC/127/18/09/662

Your Ref:

September 19, 2018

Dear Dr. Robert Downing

RE: UVRI REC review of Protocol titled **"Uganda Arbo Viral Infection Study (AVI)"**

Thank you for submitting the response to queries addressed to you by UVRI REC.

This is to inform you that your response dated August 10, 2018 has been reviewed and met the requirements of the UVRI REC.

UVRI REC annual approval has been given for you to conduct your research up to September 19, 2019. Annual progress report and request for extension should be submitted to UVRI REC prior to the expiry date, to allow timely review.
The reviewed and approved document include;

Document	Version
Uganda ArboViral Infection Study (AVI)	V2.0
Appendix A (consent forms A-H)	V1.1
Appendix B (data collection templates 1-5)	V1.1
Consent forms A-H in Lugbara	V1.1
Consent forms A-H in Lukonzo	V1.0
Consent forms in E and H in K'jong	V1.0
Consent forms E and H in Luganda	V1.0

You can now continue with your study after registration with the Uganda National Council for Science and Technology (UNCST).
Note: UVRI REC requires you to submit a copy of the UNCST approval letter for the above study before commencement.

Dr. Tom Lutalo
Chair, UVRI REC
C.C Secretary, UVRI REC

UGANDA VIRUS RESEARCH INSTITUTE ENTEBBE
RESEARCH ETHICS COMMITTEE
APPROVED
19 SEP 2019
P. O. BOX 49, ENTEBBE
UGANDA

Uganda Virus Research Institute
Plot 51-59, Nakiwogo Road, Entebbe
P.O. Box 49, Entebbe-Uganda
Tel: +256 414 320 385 / 6
Fax: +256 414 320 483
Email: directoruvri@uvri.go.ug

UNHRO
Uganda National Health Research Organisation

REPUBLIC OF UGANDA

Our Ref: GC/127/662
Your Ref:
February 07, 2023

To: Prof. Emma Thomson,

Re: Application Title**: "Uganda Arboviral Infection Study (AVI)."**

Type: [✓] Protocol Amendment

Thank you for submitting your Amendment report for the above study dated February 22, 2023 to the UVRI Research Ethics Committee (REC).

This amendment was reviewed and met the requirements of the UVRI Research Ethics Committee. UVRI REC approval has been given for you to continue with the proposed amendment.

The reviewed and approved amendments are;

1. To recruit more patients into acute febrile illness study and to extend testing in this group to include participants older than two years and use both HTS and specific PCR and serology testing for CCHFV.
2. To carry out an additional serosurvey in human participants (approximately 5400 participants) and domestic animals (up to 10,500 animals) and a tick survey from 21 sites across Uganda with in-depth interviews to assess risk of exposure to CCHF and other emerging viruses. These sites include Kalangala, Mayuge, Mubende, Masindi, Kaabong, Tororo, Namutumba, Lira, Soroti, Sironko, Kasese, Bundibugyo, Kisoro, Mbarara, Lyantonde, Nebbi, Arua, Kampala, Gulu, Moyo and Nabilatuk
3. Added languages: Acholi, Alur, Ateso, Nyakaramojong, Lhukhonzo, Lubwisi, Luganda, Lugbara, Lugisu, Lwo, Ma'di, Nyole/Lunyole, Nyoro/Runyoro, Rufumbira, Runyankole, Soga/Lusoga.

4. **The following Forms have been added:**

- **Consent form A (febrile adult) _V3 English**
  - Changed from version 2 to version 3
  - Added 'febrile' to the title to make it more expressive, as well as added the information that for an adult who is unable to provide consent for this moment, a next of kin can sign it.
  - For the new study part, changed the people to be included to 2000 in total.
  - Slightly changed the wording of the beginning of the description, without a change in content.
  - Increased expense allowance from 5000UGX to 15000UGX due to increased costs and high inflation. As well as for follow-up visits from 10000UGX to 20000UGX.
  - Additional contact people were added (Dr Stella Atim and Marina Kugler)
- **Consent form B (parental.guardian permission febrile child)_V3 English**
  - Changed from version 2 to version 3
  - Added 'febrile' to the header to make it more expressive.

- Removed the addition ‘for adults unable to provide consent’, which is now covered in consent form A, by a next of kin assent form.
- Slightly changed the wording of the beginning of the description, without a change in content.
- Participants to be included in the new study part updated to 2000 in total.
- Increased expense allowance from 5000UGX to 15000UGX due to increased costs and high inflation. As well as for follow-up visits from 10000UGX to 20000UGX.
- Additional contact people were added (Dr Stella Atim and Marina Kugler)

- **Consent form C (febrile child assent) _V3 English**
  - Changed from version 2 to version 3
  - Added ‘febrile’ to the title to make it more expressive.
  - Participants to be included in the new study part updated to 2000 in total.
  - Changed outline to start with blood taking first and HIV test later.
  - Removed part about the second visit and its travel compensation, because it is included in the parent/guardian permission form.
- **Consent form D (healthy adult) _V3 English**
  - Changed from version 2 to version 3
  - Explanation of the new study part, including the new participant number of up to 5250 participants.
  - Including a possible follow-up in one year.
  - Increased expense allowance from 5000UGX to 15000UGX due to increased costs and high inflation.
  - Additional contact people were added (Dr Stella Atim and Marina Kugler)
- **Consent form E (adult biobank) _V3 English**
  - Changed from version 2 to version 3
  - Only small changes in wording, without content change.
  - Adults which are temporarily unable to provide consent, will not be covered under a guardian permission consent, but rather by the regular consent form (E, adult biobank), with a signature by a next of kin.
- **Consent form F (parental.guardian permission biobank)_V3 English**
  - Changed from version 2 to version 3
  - Only small changes in wording, without content change.
  - Adults which are temporarily unable to provide consent, will not be covered under a guardian permission consent, but rather by the regular consent form (E, adult biobank), with a signature by a next of kin.
- **Consent form G (animal sampling) _V3 English**
  - Changed from version 2 to version 3
  - Only small changes in wording, without content change.
  - Addition of tick collection from animals and environment
  - Increased expense allowance from 5000UGX to 15000UGX due to increased costs and high inflation.
  - Additional contact person was added (Dr Stella Atim)
- **Consent form I (FGD and IDI) _V1 English**
  - A new form, specifically for focus group discussions and in-depth interviews
- **Consent form J (parental.guardian permission healthy child)_V1 English**
  - Consent form B (parental.guardian permission febrile child) works as a starter and is adjusted for parental/guardian permission for a healthy child.
  - Explanation of the new study part, including the new participant number of up to 5250 participants.
  - Including a possible follow-up in one year.

- Increased expense allowance from 5000UGX to 15000UGX due to increased costs and high inflation.
- Additional contact people were added (Dr Stella Atim and Marina Kugler)

- **Consent form K (healthy child assent) V1 English**
  - Consent form C (febrile child assent) works as a starter and is adjusted for healthy child assent.
  - Participants to be included in the new study part updated to 5250 in total.
  - Including a possible follow-up in one year.
- **Consent form L (parental. Guardian permission FGD and IDI)_V1 English**
  - Adjusted from Consent Form 1 to cover the parental or guardian permission for a child to take part in the focus group discussion.
- **Consent form M (FGD and IDI child assent) V1 English**
  - Adjusted from consent forms I and K, to create a consent form for a focus group discussion for children.
- **Consent form N (media consent form) _V1 English**
  - A consent form is made to ask for consent to use participants' pictures for future use.
- **Focus group discussions and in-depth interviews.**
  - New document to describe the big questions and provide guidance for the focus group discussions and in-depth interviews.
- **Questionnaire_AVI_V3**
  - Adjusted from previous versions.
  - New questions include:
    - Exact GPS location
    - Recruitment group
    - Questions to estimate the socioeconomic status (furniture, fuel, mobile phone, internet access, bank account)
    - Visiting a national park
    - Consume unpasteurized mild or cheese.
    - Consume dairy products.
    - Tested positive for COVID-19
    - Vaccine for Covid-19
    - Fever in the last 3 weeks
    - Seek advice or treatment, when and where?
    - Hypothetical scenario for seeking advice and treatment for fever.
    - Sleeping close to animals
    - Picking engorged ticks
- **Questionnaire_INCIDENCE_V1**
  - New document to assess risk of exposure to a viral haemorrhagic fever in the 21 days.

You can continue with your study and remember to notify Uganda National Council for Science and Technology (UNCST).

Yours Sincerely,

Dr. Tom Lutalo
Chair, UVRI REC
C.C File, UVRI REC

Uganda National Council for Science and Technology
(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS 2485 **4th December 2018**

Dr. Emma Thomson
Principal Investigator
C/o Uganda Virus Research Institute
Kampala

Dear Dr. Thomson,

Re: Research Approval: Uganda ArboViral Infection Study (AVI)

I am pleased to inform you that on **03/12/2018**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **03/12/2018** to **03/12/2023**.

Your research registration number with the UNCST is **HS 2485.** Please, cite this number in all your future correspondences with UNCST in respect of the above research project.

As Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the research protocol or the consent form (where applicable) must be submitted to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local IRC for review with copies to the National Drug Authority.
4. Unanticipated problems involving risks to research subjects/participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST review.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. An annual progress report and approval letter of continuation from the REC must be submitted electronically to UNCST. Failure to do so may result in termination of the research project.

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
P. O. Box 6884
KAMPALA, UGANDA

COMMUNICATION

TEL: (256) 414 705500
FAX: (256) 414-234579
EMAIL: info@uncst.go.ug
WEBSITE: http://www.uncst.go.ug

Below is a list of documents approved with this application:

	Document Title	Language	Version	Version Date
1.	Research proposal	English	3.0	November 2018
2.	Consent forms	English, Ngakirimojong, Lugbara and Lukonzo	2.0	November 2018
3.	Voluntary participation: child (Aged 8 – 18 years) assent form	English, Ngakirimojong, Lugbara and Lukonzo	2.0	November 2018
4.	Data record forms	English	1.1	N/A

Yours sincerely,

Isaac Makhuwa
For: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Copied to: Chair, Uganda Virus Research Institute, Research Ethics Committee

LOCATION/CORRESPONDENCE

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Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS 2485 **1st December 2023**

Dr. Emma Thomson
Principal Investigator
C/o MRC-Uganda
ENTEBBE

RE: UGANDA ARBO VIRAL INFECTION STUDY (AVI)

This is to inform you that on 1st December 2023, Uganda National Council for Science and Technology (UNCST) reviewed the progress report and application for renewal and approved the continuation of the above study. UNCST granted continuing approval valid until **3rd December 2028.**

If, however, it is necessary to continue with the study beyond the expiry date, a request for continuation should be made to the Executive Secretary, UNCST

Yours sincerely,

Beth Mutumba
FOR: EXECUTIVE SECRETARY

Cc: The Chairperson, Uganda Virus Research Institute-Research Ethics Committee

LOCATION/CORRESPONDENCE

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Telephone: General Lines: 256 – 417 - 712260
Permanent Secretary's Office: 256 – 417 - 712221
Toll Free 0800100066
E-mail: ps@health.go.ug
Website: www.health.go.ug

IN ANY CORRESPONDENCE ON
THIS SUBJECT PLEASE QUOTE No. ADM. 140/269/01

THE REPUBLIC OF UGANDA

Ministry of Health
P. O. Box 7272
Plot 6, Lourdel Road,
Wandegeya
KAMPALA
UGANDA

23rd November 2023

The Chief Administrative Officer

…… District

FAO: The District Health Officer,

……..……..District

RE: Introduction of the Team Undertaking the Study of ArboViral Infection (AVI) in Uganda

I'm writing to introduce to you the team undertaking the study of ArboViral Infection in Uganda. The Uganda Virus Research Institute (UVRI) with partners from the London School of Hygiene and Tropical Medicine (LSHTM), and MRC-University of Glasgow Centre for Virus Research (CVR) from the United Kingdom are conducting a study to map the risk of Crimean-Congo Haemorrhagic fever virus (CCHFV) and other arboviruses in Uganda to inform control strategies that will mitigate the impact of these diseases on public health.

The study will be conducted in the districts of Kalangala, Kasese, Kisoro, Arua, Soroti, Kaabong, Namutumba and the Kampala Capital City Authority (KCCA), commencing with the social science component to understand socio-behavioral factors associated with the disease and subsequent human, animal and tick surveys. The study was cleared by the UVRI Science and Ethics Committee and by the Uganda National Council of Science and Technology.

The purpose of this letter is therefore to notify you about the study and request you to render the team all the necessary support to meet the study objectives.

Thank you. I remain, Yours Faithfully

Commissioner,
Integrated Epidemiology Surveillance and Public Health Emergencies
Ministry of Health

cc The Resident District Commissioner

Appendix C

Consent forms

VOLUNTARY PARTICIPATION: CONSENT FORM I
FOCUS GROUP DISCUSSION and IN-DEPTH INTERVIEW
ADULT FORM

STUDY TITLE: An investigation into the transmission and total cases of Crimean-Congo haemorrhagic fever (CCHF) in Uganda

PRINCIPAL INVESTIGATOR: Dr Emma Thomson
FUNDER: Wellcome Trust and Medical Research Council

This study is part of a bigger study in understanding the transmission risk of Crimean-Congo haemorrhagic fever (CCHF) in Uganda. The study is being conducted by the Uganda Virus Research Institute (UVRI) and the MRC-University of Glasgow Centre for Virus Research, UK (CVR).

CCHF is present in Uganda, with sporadic outbreaks and deaths, but also high exposure in the community has been detected. The study aims to understand better, how you and your community might be exposed to the disease and how much knowledge is available about CCHF. This will guide us through our further research and will help us to understand better how to prevent people from getting ill with this infection.

We will include people from all over Uganda, from different regions and districts. We will ask about different life experiences and life situations, to help us to understand how the infection is transmitted in your community.

BEING IN THE STUDY
If you want to be in the study, you should understand that:

- Being in the study is up to you.
- All participation in this study is voluntary. You are free to decide if you want to take part. You are free to withdraw at any time. This will not affect you in any way, now or in the future.

If you decide to take part in this study, several things will happen:
- You will be asked to give consent to take part in the study. The consent will be written, or thumbprint on a form and you will be given a copy of it.
- You will be requested to take part in a **group discussion/individual interview** (circle applicable) with our study team. This interview will be tape-recorded to be able to get the interview clearly and possibly present parts of it for the wider scientific community (cross if not applicable).

RISKS
We do not anticipate any risks from taking part in this study. Anything you tell us as part of the study will be kept confidential. We will not discuss the things you tell us. We will not discuss the names of participants in anything we say or write.

BENEFITS

There are no individual benefits for you in taking part in this study. However, by participating you will help us improve our understanding of the transmission of CCHF. If you decide to enrol on this study, you will be provided with a sum of fifteen thousand Ugandan Shillings to compensate you for your time spent during the interview.

PRIVACY

All our research records are stored securely in locked cabinets and password-protected computers. You will be assigned a unique identifier number that will be used instead of your name. The consent form and any other main list with your name and unique identifier will be securely kept in a restricted locked cabinet at the research site in the Uganda Virus Research Institute. Your name will never be used on any presentation or publication from this study. The people who may review your records include study investigators, Research Ethics Committee (UVRI-REC), study collaborators, funders, and research staff at the site. Short parts/few sentences might be used for presentations to the wider scientific community. By signing the assent/consent form, you authorise this access.

COSTS

You will not need to pay anything for being in this study. You will not be paid for being in the study.

QUESTIONS

If you want to ask questions about this project, please talk to the study team or contact Dr Stella Atim or Marina Kugler on the study phone at +256 706 486 207.
If you have questions about your rights being in this study, please contact Dr Tom Lutalo, Uganda Virus Research Institute Research Ethics Committee; Office phone 041-4321 962.

This study has been approved by Uganda Virus Research Institute Research and Ethics committee.

If you sign or make your thumbprint on this form it means that you have read what it says, or that somebody has read it to you and that you agree to participate in the study.
We will give you a copy of this letter.

_____	_____	
Participant signature or thumbprint	Print name	Date
_____	_____	
Study staff signature	Print name	Date
_____	_____	
Witness signature*	Print name	Date

*required where the subject is illiterate

VOLUNTARY PARTICIPATION: CONSENT FORM D

HEALTHY ADULT (aged 18 years and older) ASSENT FORM

Many diseases including malaria and typhoid cause an illness with fever. Sometimes illness with fever has a different cause. We would like to identify all the common diseases that can cause this kind of illness in Uganda, so we can improve our tests and give people better medicines in the future. To do that, we want to find out which diseases have people had in their life, by testing their blood for past infections that they don't have anymore.

The Uganda Ministry of Health (UMoH) and doctors from the Uganda Virus Research Institute (UVRI) and the MRC-University of Glasgow Centre for Virus Research in the United Kingdom (CVR) want to learn what is causing illness with fever in the area where you live so that better tests and the right treatment can be given in future. We are conducting a Uganda wide study in 21 districts with up to 5250 participants, to compare regions and identify risk areas. We also plan a follow-up of a subgroup, to see changes in infections occurring in one year. If you join this study, a trained health worker will take blood samples from you, and the blood will be tested to see if you were exposed to different viruses in the past.
This research has been sponsored by the Medical Research Council (MRC) and the Wellcome Trust.

BEING IN THE STUDY
If you want to be in the study, you should understand that:

- Being in the study is up to you.
- Being in this study may help people who are sick by identifying what diseases are causing illness in this area.
- Being in this study may help us stop other people from getting sick in the future.
- Being in this study will involve very little pain or risk to you.
- Being in this study will require that a trained health worker will ask you some questions today and collect a sample of your blood, urine, and a throat swab.
- Being in this study will mean that a trained health worker will offer you a finger-prick test for HIV. You do not have to accept this test. If the test is positive, you will be referred to a local clinic for treatment.
- A study investigator may visit your home to take note of its location.
- You will not be able to access the results of the tests done in the study.
- If you are in the study but later want to stop, you may ask to stop being in the study.
- You might be asked to join a follow-up in one year, where again questions are asked, and a blood sample will be taken.

WHAT YOU NEED TO DO IF YOU JOIN THE STUDY
If you want to be in the study, the study researcher will:

- Ask you today about how you are feeling today.
- Take a sample (1-2 teaspoons total) of blood with one needle in your arm today.
- Offer you a finger-prick test for HIV today.

BENEFITS

The benefit to you from being in this study is that you may help us find out what is causing people to get sick in this area. The study may help us to prevent sickness in the future. The study may also help us to provide better treatments for illness with fever in the future.

If you decide to enrol into this study, you will be provided with a sum of fifteen thousand Ugandan Shillings to compensate you for your time spent during the clinical assessment and questionnaire.

RISKS

The risks to you from being in this study are small. You will feel a pin prick when samples are taken. The hurt will be over quickly. It may leave a small bruise.

PRIVACY

We will keep the facts about you as private as the law allows. Doctors or researchers from the Uganda Virus Research Institute (UVRI) or the MRC-University of Glasgow Centre for Virus Research (CVR) may look at the information we collect and the questions we ask you. When we tell other people about the results of this work, they cannot find out your name.

COSTS

You will not need to pay anything for being in this study. You will not be paid for being in the study.

QUESTIONS

If you want to ask questions about this project, please talk to the study team or contact Dr Stella Atim or Marina Kugler on the study phone at +256 706 486 207.
If you think you have been harmed or have questions about your rights being in this study, please contact Dr. Tom Lutalo, Uganda Virus Research Institute Research and Ethics Committee; Office phone 041-4321 962.
This study has been approved by Uganda Virus Research Institute Research and Ethics committee.
If you sign or make your thumbprint on this form it means that you have read what it says, or that somebody has read it to you and that you agree to participate in the study. We will give you a copy of this letter.

_____	_____	_____
Participant signature or thumbprint	Print name	Date
_____	_____	_____
Study staff signature	Print name	Date
_____	_____	_____
Witness signature*	Print name	Date

*required where the subject is illiterate

FUTURE USE OF SAMPLES: CONSENT FORM E

ADULT (aged 18 years and older, including next of kin, in case of an adult unable to provide consent)

The Uganda Virus Research Institute and the MRC-University of Glasgow Centre for Virus Research would like to save your blood and/or saliva and/or throat swab and/or urine samples that are left over after we do the tests for this research study. In case you are included in the animal studies, we would like to keep the blood samples of your animals that are left over after we do the test for this research study. We want to ask you if we may keep the left-over samples in Entebbe or in the United Kingdom for additional research studies in a 'Biobank'. We plan to use these samples for studies we hope to do in the future. Any future testing will be related to diseases caused by viruses or other pathogens such as bacteria that cause people to become unwell with a fever. We will store these samples with some data about you and/or your animals, such as age, sex, and other information collected during the study. However, we will NOT put your name on the samples, and there will be no way to know whom the samples came from. Thus, we will not be able to report back any test results to you. You can decline to let us store your samples and still be in the study. If you do not agree to let us keep your samples, we will treat you just as well as when you do agree. We will store these samples for up to 25 years.

I agree that my left-over samples may be saved for additional research:

_____	_____	_____
Name (please print)	Signature/thumbprint	Date

I do not agree to have my left-over samples saved for additional research:

_____	_____	_____
Name (please print)	Signature/thumbprint	Date

Study staff signature:

_____	_____	_____
Name (please print)	Signature	Date

Witness signature*:

_____	_____	_____
Name of Witness (please print)	Signature	Date

*required where the subject is illiterate

Appendix D

Surveys, topic guides, codebook

Demographics – AVQ Qualitative study

Record ID	
In which group are included?	☐ Community leaders FDG ☐ Men FDG ☐ Women FDG ☐ Children FDG ☐ In-depth interview ☐ NA
Which district do you live in?	☐ Kampala ☐ Kalangala ☐ Kasese ☐ Soroti ☐ Aura ☐ Kaabong
Which village do you live in?	
What is your age in years?	
What is your sex?	☐ Female ☐ Male ☐ NA
What is your religion?	☐ Christian ☐ Muslim ☐ None ☐ Other, which:
What is your education level?	
What is your tribe?	☐ Baganda ☐ Bassese ☐ Batooro ☐ Bakonjo ☐ Lugbara ☐ Iteso ☐ Kumam ☐ Karamojong ☐ Others, **which:** ☐ NA

What is your occupation?	
Do you have any other sources of income? If yes, which one?	
What is your marital status?	
Do you have children? If yes, how many and which age range are they?	
Who are you living with? (partner, children, grandchildren, friends, maid)	
What is your housing situation? (eg flat, house)	
Do you have animals, and if yes, what animals? (dogs, cows, cats, rabbit)	
Do you own land? (size, area, environment eg forest)	

FOCUS GROUP DISCUSSION OUTLINE

This is the outline for the focus group discussions which are part of the AVI study in Uganda.

1st part: Icebreakers/Social demographics
(individual before the group meeting, enter in REDCap)

- Please tell me a few things about yourself?
  (sex, age, religion, education, tribe, housing situation, household composition, marital status, number of children, village/district, occupation, other sources of income, living environment (swamp, around animals, number of animals), landownership)
- How is the living environment for people in your community? (swamp, slum, houses, high grass, football pitch)

2nd part: Knowledge and Understanding about ticks and CCHF

- Do you know of any diseases which can cause fever, abdominal pain and various bleeding? (bleeding, Ebola, others, transmitted through animals)
- Have you every heard about CCHF, what do you know about it? (causes, transmission, cases in your community, treatment)
- What do you know about ticks? (life cycle, hosts, feeding, affecting humans, danger, concerns, transmission of diseases, how)

3rd part: General information on ticks and CCHF (explain to the participants)

- Ticks are feeding on blood. The blood can be from farm animals, wild animals and also humans. (show pictures of fully fed and non fed as well as nymph, explain **lifecycle**)
- Ticks can transmit diseases, including bacteria, parasites and viruses.
- Crimean-Congo Heamorrhagic fever is a disease caused by the virus CCHFV, which can be transmitted by ticks, but also by direct contact with infected animal blood and tissue.
- CCHF starts with fever, muscle ache and eye soreness, and can further include abdominal pain, bleeding into the skin and other fatal symptoms which lead to death in around 30% of the cases. (pictures)
- There is no vaccination of the disease, which is why prevention is so important.

4th part:
4.1: Individual Behaviour and Perception

- Have you or anyone you know had the disease diagnosed? (symptoms, transmission, bias, what happened in the community)
  - What would you do if you would experience symptoms like fever with abdominal pain and bleeding into the skin? (who do you go to, health seeking)
  - How do you think having and/or surviving CCHF could change your life? (fear, socio, economic, physical impact, stigma)
- Have you or anyone you know ever had a tick bite? (suspicious of an insect on/attached to the skin, how many times, which categories, age groups, area, why?)
  - What do people do when they had a tick bite? (how, removing, disposal)
  - What symptoms do they experience and how do they treat the symptoms? (itchiness, wounds infections, fever, redness, sick, facility, pharmacy, creme)
- What activities make you interact with animals? (handeling, milking, feeding, treatment, cuddling, spraying, change materials used)

    - Where do you keep your animals? (garden, farm, shelter, in the house)
    - What do you do in case your animals have ticks on them? (picking, spraying, dipping)
    - How do you slaughter your animals? (abattoir, technique)
- What activities make you interact with wild animals? (hunting, ticks)
- How does the community interact with rodents? (many around, traps, killing)
    - Have you seen ticks on them?

4.2: Environmental influences

- How often do you see ticks in your area, where do you find them and which time of the day do you see them? (garden, animals, grass, morning, afternoon, evenings)
    - Do you see a trend during the year and/or within the past years in the presence of ticks?
        - What do you think has caused these trends? (seasons, climate change, landuse, flooding)
    - What are you currently doing to control ticks in your community? (acaricides, changes, grass cutting, burning)
        - What are the various sources of help available for people in the community to control ticks? (sources, government, organisations, agricultural extension officers)

4.3: Cultural Practices and Believes

- Are there local cultural believes that influence the communities interactions with animals, their blood and possible ticks? (how)
    - Are there specific rituals or activities that involve contact with animals or their animal products like blood or tissues? (slaughtering, traditional medicine)
- Are there local cultural practices which affect the use of protective measurements with patients or deceased? (burials, prayers)

5th part: Recommendations

- Do you have anything in mind we haven't touched yet?
- What would you want to be done to prevent and control the tick burden? (personal, community, policies)
- What would you want to be done to prevent CCHF? (personal, community, policies)
- Do you have any questions or comments for us?

Codebook

Theme	Parent code	Child Code	What it covers	Possible Overlap
Theme 1: Knowledge and awareness of haemorrhagic diseases			This theme is part of the previous knowledge part of the topic guide. These are answers which are given before the disease and disease transmission are explained by the researcher. This part is specifically to heamorrhagic diseases.	
	CCHF knowledge	-	Anything mentioned to CCHF (including, symptoms, spread, diagnosis, transmission, source of information, no awareness)	
	Ebola knowledge	-	Anything mentioned to Ebola (including, symptoms, spread, transmission, source of information)	
	Other knowledge	-	Any other diseases which are mentioned when asked about the sympoms and causes of haemorrhagic fevers. This includes diseases which are not classified as haemorrhagiceg disease like Cholera or Malaria. But also other diseases like Yellow fever or Marburg fever.	
Theme 2: Knowledge of ticks			This theme is part of the previous knowledge part of the topic guide. These are answers which are given before the disease and disease transmission are explained by the researcher. This part is specifically to ticks.	
	Dangers of ticks	-	Are people aware of any dangers of ticks. This includes all species, animals and humans, as well as when not specified.	
	Dangers of ticks to specifically humans	-	When the danger is specifically mentioned to be to human, this is additionally coded.	
	General awareness	-	This includes the general awareness or not, of ticks.	
Theme 3: Community cases CCHF			The community cases theme includes every actual real life scenario people talk about. Eg suspeced cases as well as diagnosed cases of CCHF or a disease looking similar. This also includes people telling stories of people with symptoms looking similar to the once on the pictures. Or it includes when no such symptom was every seen.	
	CCHF diagnoses and treatment	-	How where these people diagnosed and/or treated?	Theorethical diagnosis path -> Other; Hospital procedure for suspected CCHF case
	CCHF symptoms	-	Symptoms presented by the people mentioned of suspected or diagnosed cases.	Theorethical symptoms to cause suspection -> Other; Hospital procedure for suspected CCHF case
	CCHF transmission	-	What is believed caused the disease, are other people infected or followed up, was there a study conducted to investigate more?	
	Community cases CCHFV general	-	This includes when people talk about an actual case of CCHF, as well as there are no case identified or the community is not sure about any	
Theme 4: Health seeking behaviour			Health seeking behaviour includes anything to do with what people do when they feel sick. This includes self medication, pharmacies, traditional healer or herbalists, hospitals and medical practices.	
Theme 5: Changes during and after sickness of CCHF			This includes how people think they and their families lifes would change if they or someone around them would be infected with CCHFV, gets sick and recovers. For example financial worries, behavioural changes like avoiding animals or stigmas from the communities.	
Theme 6: Tick bites			The tick bite theme records in detail if tick bites occure in the community, and what people do with a tick bite. This should record the experiences of the people but can also a theoretical scenario, what would they do or what they think. This includes animals and humans.	
	Most affected by tick bites	In animals - most affected	Are there animals which are more prone to tick bites (species, bread, different location)? Where do the ticks bite the animals (location, ear, utter)?	
		In humans - most affected	Are there any age groups more prone to tick bites (children, teenager), or are there any occupations where you see more tick bites (herdsman, abattoir)? Where do you find the ticks bite usually (head, legs)?	
	Removal and discarding of ticks	Animals and unspecified - removal	How to remove and discard the ticks which bit an animal. This can be spraying and plucking it off. As well as burning or throwing it away. If it is spoken general and could mean from an animal or human it is included here.	
		Humans (specified) - removal	How to remove and discard the ticks which bit a person.	This is only if it is mentioned specifically. If unsure, add to -> 'animals and unspecified'.

	Symptoms and treatment of tick bites	Animals and unspecified - Symptoms and treatment	Which symptoms people see in their animals or believe they experience with a tick bite, as well as how they treat it. (eg diseases, milk volume) This also includes if people talk about general symptoms and treatment of tick bites but it is not clear if it occured in animals or humans. If it is spoken general and could mean from animals or humans it is included here.	
		Humans (specified) - Symptoms and treatment	Which symptoms people experience after a tick bite (swelling, itching, fever), and how and if they treat any symptom (crème, pharmacy).	This is only if it is mentioned specifically for humans. If unsure, add to -> 'animals and unspecified'.
	Tick bites community cases	Animals and unspecified - tick bites	Are tick bites common or do they occur in this community on animals? If it is spoken general and could mean tick bites are found on animals and humans it is included here.	
		Humans (specified) - tick bites	Do tick bites occure on humans?	Only when someone specifies that they are talking about tick bites on humans. If unsure, add to -> 'animals and unspecified'.
Theme 7: Possible risks for tick bite			A tick bite is a clear risk for CCHF. In this theme we record possible behaviour, which could lead to a tick bite or could be an increased risk for tick bites in the community.	
	Animals	Activities with animals	Anything people do in close contact with animals, like milking, cuddling, cleaning the krall, spraying, and others.	
		Animal keeping	How do people keep their animals? Eg in a krall, inside the house, in a shelter, how and where are the animals grazing.	
		Animal tick control	What do people do to prevent tick bites. etive measurememnts (Acaricides (plus resistance), Help form organisations/government)	This includes purely preventive measurements against tick bites on animals. When they talk about spraying to remove the ticks on the animals, that is -> 'removal and discarding of ticks', 'Animals and unspecified'
		Cultural practices	Anything culturally for the community or area which is related to a close contact to animals. This includes for example bride prices of animals and their preparations, or a close contact due to a believe.	This should only be if defined by the community as cultural, if not defined, this should just go to -> 'animals', 'activities with animals'
		People handling animals	Mentioned people which do the above activities with animals, eg groups like age groups or occupations. Also separations like some own the animals but others look after them on a day to day basis.	
	Environment factors	District-wide environment	To code district wide, this will include the environment distric-wide. This is on a large scale, eg forests, national parks, semi-arid, boardering districts and counties and more.	
		Environment tick control	What do people do to control ticks in the environment (grass burning, cutting grass) and is there any help from organisations/governemnt?	This includes only the control of ticks in the environment, not on animals. -> 'animals', ' Animal tick control'
		Living environment	This codes for the living environment in a small scale, which means just around their houses or within their community. For example bushy areas, gardens, animals, trees, hills and valleys a river or a lake, the househing structures, playing grounds, congestions and other factors.	
		Presence of ticks	Do people see ticks in the environment? (includes abundancy, time of the day, differences with seasons, differences due to climate change, locations eg grass, bushes)	
	Rodents	-	Anything regarding rodents around the houses, contact with rodents or similar.	
	Wild animals	-	Anything regarding wild animals, including hunting, eating wild meat, and more.	
Theme 8: Possible risks for direct transmission			CCHF can also be transmitted directly from viremic animals. In this theme we code for possible contact with animal products and a possible risk for direct transmission.	
	Cultural and religious practices	Caring for sick or deceased person	This should include any mentioning of believes around sick or deceased people. Eg use of PPE, direct contact, burials.	
		Handling animal products	Anything culturally for the community or area which is related to handling animal products. This includes for example animals slaugthered at traditional healer or practices due to believes.	This should only be used if defined by the community as cultural, if not defined, this should just go to eg -> 'slaughtering' or 'eating and selling animal products'
	Eating and selling animal product	Blood	Mention everything in regards to blood drinking and eating (eg raw, cooked, prepared, specific times, who).	
		Meat	Anthing in regards of eating meat from animals. This includes reports of meat from sick animals as well as if meat is sold because then it will be eaten from other people. As well as preparation frequency.	
		Milk	Anything in regards of drinking or eating milk products, including preparation.	
	Eating ticks	-	Mentioning of eating ticks, includes preparation and frequency.	
	Slaughtering	People	Who slaughters, eg muslim, elderly people	
		Location	Where the slaughtering happens, eg at home or in the abattoir.	
		PPE	Anything to PPE use while slaugthering	

	Method	Includes everything to the process, and how is done, eg blood collection, hold by multiple people.
Treatment and diagnosis of animals	-	Inlcudes anything to do with sick animals, if and how they can be diagnosed and treated. This includes not only sick with CCHF, but also other diseases or unspecific fevers.
Theme 9: Other		This includes other codes which come out in the transcripts.
District procedure for suspected CCHF case	-	Mentioned steps undertaken in a district when case is suspected in humans as well as in animals. This includes the OneHealth aspect.
Hopsital procedure for suspected CCHF case	-	This includes what would happen in a hopsital with a suspected case (eg trained staff, samples, diagnosis, isolation)
Theme 10: Recommendations and ideas		We received a lot of recommendations and ideas for prevention of tick bites and CCHF, which are coded within this theme.
Adherence to recommendations	-	Anything to do with the wish that more people should adhere to the recommendations already given, possible with punishments as well.
Feedback	-	Includes the wish for feedback from this research directly to the communitites.
Research	-	This includes the wish for more research in the field.
Sensitisation and awareness	-	Anything to do with the wish or recommendation for more sensitasation and awareness within the community.
Supply and focus by the government	-	Mention anything where people ask for more help or supply from the governemnt.
Theme 11: Unknown code		Anytime the text doesn't fit to anything of the above.

HOUSEHOLD NUMBER
AVH -

Arboviral infection study DATA RECORD SHEET

HOUSEHOLD SURVEY AVI study

DATE .....

Name of study team member completing this questionnaire .....

In which **district** do you live? .....

In which **subcounty** do you live? .....

In which **village** do you live? .....

Record GPS location: Latitude: ..... Longitude: .....

1. Is this household from the A (first) list or the B (second) list? (The A list are the first 10 households which will always be visited, and fully recruited if eligible; The B list are additional 10 households, which can be used to recruit more households if some were not eligible in the A list, as well as to fill up all age groups so that in each age group, 10 participants are recruited) ○ A ○ B	2. Is the household fully recruited? (Fully recruited means there will be no selection of specific age groups. One person will be selected and recruited from all age groups available.) ○ Yes ○ No
3. Is everyone in the household healthy? (No signs of fever, anaemia, or severe malnourishment) ○ Yes ○ No, please specify	4. Is everyone in the house willing to participate in the study if selected? ○ Yes ○ No, please specify ....

The household can be included in the study. Please carry on and complete the questionnaire.

The household is NOT ELIGIBLE for participation in the study. Please STOP - the household cannot be recruited. Please give detailed information on why the household will not be participating.

.....

5. Record the total amount of people living in this household. (everyone who sleeps several nights of the week in this house, including only at weekends; includes nonfamily household members like maids or helpers; but a minimum stay of one year is needed; all ages - also below 2 and newborns) .....

6. How many people between 2 and 14 years old live in this household? ----------	7. How many people between 15 and 27 years old live in this household? ----------
i. Child 1: record age and sex ----------	i. Youth 1: record age and sex ----------
ii. Child 2: record age and sex ----------	ii. Youth 2: record age and sex ----------
iii. Child 3: record age and sex ----------	iii. Youth 3: record age and sex ----------
iv. Child 4: record age and sex ----------	iv. Youth 4: record age and sex ----------
v. Child 5: record age and sex ----------	v. Youth 5: record age and sex ----------
vi. Child 6: record age and sex ----------	vi. Youth 6: record age and sex ----------
vii. Child 7: record age and sex ----------	vii. Youth 7: record age and sex ----------
viii. Child 8: record age and sex ----------	viii. Youth 8: record age and sex ----------
ix. Child 9: record age and sex ----------	This is the randomly selected person to be sampled in the age group 15 - 27 years. The number of the randomly selected person is: ----------
x. Child 10: record age and sex ---------- This is the randomly selected person to be sampled in the age group 2 - 14 years. The number of the randomly selected person is: ----------	

HOUSEHOLD NUMBER

AVH -

8. How many people between the ages of 28 and 40 live in this household? ..... i. Adult 1: record age and sex ..... ii. Adult 2: record age and sex ..... iii. Adult 3: record age and sex ..... iv. Adult 4: record age and sex ..... v. Adult 5: record age and sex ..... vi. Adult 6: record age and sex ..... vii. Adult 7: record age and sex ..... viii. Adult 8: record age and sex ..... ix. Adult 9: record age and sex ..... This is the randomly selected person to be sampled in the age group 28 to 40 years. The number of the randomly selected person is:	9. How many people above the age of 41 live in this household? ..... i. Elder 1: record age and sex ..... ii. Elder 2: record age and sex ..... iii. Elder 3: record age and sex ..... iv. Elder 4: record age and sex ..... v. Elder 5: record age and sex ..... vi. Elder 6: record age and sex ..... vii. Elder 7: record age and sex ..... This is the randomly selected person in the age group above 41 years. The number of the randomly selected person is: .....

Questions to estimate your socioeconomic status.

10. Does your household have…
    - electricity? ○Yes ○No
    - a radio? ○ Yes ○No
    - a cassette/CD/DVD player? ○ Yes ○No
    - a sofa set? ○ Yes ○No
    - a cupboard? ○ Yes ○No
    - a television? ○ Yes ○No

11. Does any member of your household…
    - …own a watch?

      ○ Yes ○ No
    - …have a bank account?

      ○ Yes ○ No

12. What type of fuel does your household mainly use for cooking?

○ Wood
○ Charcoal
○ Other fuel type

13. What is the main material of the ……. in your dwelling (residence)?
    - floor ○Cement ○ Other material
    - exterior wall ○Burnt bricks with cement ○Other material
    - roof ○Thatch/palm leaf ○Other material

Thank you for your time! We will continue with the selected people for the individual survey and blood collection.

Arboviral infection study DATA RECORD SHEET

STUDY NUMBER
AVP -

QUESTIONNAIRE AVI study

Household ID .....

DATE .....

Name of study team member completing this questionnaire .....

Has the participant given written consent to participate in this study? ○ Yes ○ No

Measure and record the body temperature in degrees Celsius .....

Is the participant healthy? (no anaemia and not severely underweight) ○ Yes ○ No

In which **district** do you live? .....

In which **subcounty** do you live? .....

In which **village** do you live? .....

The participant is eligible for participation in the study. Please carry on and complete the questionnaire.

If the participant is not eligible - please STOP - the patient cannot be recruited. Please give detailed information on why the participant will not be taking part in the study.

.....

What is the recruitment group? ○ First recruitment round ○ Follow-up

Record GPS location: Latitude: ..... Longitude: .....

A. Identification.

1. What is your **age** (years)? .....
2. What is your **sex** (M/F)? .....
3. To which **tribe** do you belong? .....
4. Were you born in the district you live now?
   ○ Yes ○ No, specify: .....
5. Have you ever lived in other district (s)?
   ○ Yes, how many?
   Please specify: .....
   ○ No
6. Have you ever lived in other country?
   ○ Yes, how many?
   Please specify: .....
   ○ No
7. Which religion do you practice?
   ○ Christianity
   ○ Islam
   ○ Other, specify: .....
   ○ None

8. What has your primary **occupation** been in the past 12 months (past year)? ○ Not employed ○ Student ○ Farm worker (crops only) ○ Farm worker (livestock only) ○ Farm worker (both crops and livestock) ○ Shop worker ○ Boda boda driver ○ Herdsman ○ Healthcare worker, specify: ..... ○ Other, specify: ..... i. How long have you been in this occupation?	9. What other job(s) have you had in your life? (please list them all) ○ Not employed ○ Student ○ Farm worker (crops only) ○ Farm worker (livestock only) ○ Farm worker (both crops and livestock) ○ Shop worker ○ Boda boda driver ○ Herdsman ○ Healthcare worker, specify: ..... ○ Other, specify: .....
10. What is the highest educational level you have reached? ○ No formal education ○ Primary school level ○ Senior school level ○ University degree ○ Other, specify:	

B. Questions about your activities over the past year.

11. Have you taken **care of a sick person** in the past 12 months (past year)? This includes contact with friends or family members who are unwell. ○ Yes, please specify ..... ○ No i. Where was the sick person cared for? ○ In hospital or a health centre ○ At home ○ Other	12. Have you **travelled** away from your home for one or more nights in the past 12 months (past year)? ○ Yes ○ No i. Where did you travel to? ..... ii. Why did you travel? (e.g. for a job, to visit family, other reasons) .....
13. Have you been to a national park or protected area in the past 12 months (past year)? ○ Yes, daily ○ Yes, weekly ○ Yes, monthly ○ Yes, few times this year ○ Yes, once ○ Never	14. Have you been inside a cave or underground in a mine in the past 12 months (past year)? ○ Yes, daily ○ Yes, weekly ○ Yes, monthly ○ Yes, few times this year ○ Yes, once ○ Never

AVP -

15. Have you consumed dairy products in the past 12 months (past year), including milk, yoghurt, butter, cheese or other products made from milk?
- Yes, daily
- Yes, weekly
- Yes, monthly
- Yes, few times this year
- Yes, once
- Never

i. If yes, which animal(s) did the dairy products come from?
- Cow
- Goat
- Sheep
- Pig
- Other, specify:.....

16. Did Have you consumed unpasteurized (raw/untreated) diary products in the past 12 months (past year), including milk, yoghurt, butter cheese or other products made from milk? Yes, weekly
- Yes, daily
- Yes, weekly
- Yes, monthly
- Yes, few times this year
- Yes, once
- Never

i. If yes, which animal(s) did the dairy products come from?
- Cow
- Goat
- Sheep
- Pig
- Other, specify:.....

17. Have you consumed raw blood in the past 12 months (past year)?
- Yes, daily
- Yes, weekly
- Yes, monthly
- Yes, few times this year
- Yes, once
- Never

i. If yes, which animal(s) did the dairy products come from?
- Cow
- Goat
- Sheep
- Pig
- Other, specify:.....

18. Have you consumed raw meat in the past 12 months (past year)?
- Yes, daily
- Yes, weekly
- Yes, monthly
- Yes, few times this year
- Yes, once
- Never

i. If yes, which animal(s) did the dairy products come from?
- Cow
- Goat
- Sheep
- Pig
- Other, specify:.....

19. Have you consumed half-cooked or lightly cooked meat in the past 12 months (past year)?
- Yes, daily
- Yes, weekly
- Yes, monthly
- Yes, few times this year
- Yes, once
- Never

i. If yes, which animal(s) did the dairy products come from?
- Cow
- Goat
- Sheep
- Pig
- Other, specify:.....

20. Have you used blood or animal products as part of a traditional medicine ritual in the past 12 months (past year)?
- Yes, daily
- Yes, weekly
- Yes, monthly
- Yes, few times this year
- Yes, once
- Never

i. If yes, which animal(s) did the dairy products come from?
- Cow
- Goat
- Sheep
- Pig
- Other, specify:.....

C. Questions about your health.

21. Do you have any **long-term illness**, such as asthma, diabetes, high blood pressure, HIV, or a cardiovascular or respiratory disorder?
- Yes, please specify
  .....
- No

22. Do you take any regular **medication**, including non-prescribed and traditional medicine?
- Yes, please specify
  .....
- No

23. Which **vaccines** have you had in your life?

- Covid-19
- Ebola
- Measles
- Mumps
- Rubella
- TB/BCGs
- Tetanus
- Diphtheria
- Polio
- Pertussis
- Rabies
- Yellow fever
- Pneumococcal
- Meningococcal
- Hepatitis B
- No vaccine
- Other

i. If other, please specify .....

ii. What was the date of your Ebola vaccine? .....

iii. What was the date of your Yellow fever vaccine?

24. Have **you ever been diagnosed** with a haemorrhagic fever? (Including Ebola virus disease, Sudan virus disease, Crimean Congo Haemorrhagic fever, Marburg virus disease)
- Yes
- No

i. If yes, please state **when** and **where** and **which illness**?
.....

AVP -

25. Have you ever been in contact with someone who was suspected of having a haemorrhagic fever illness? (Including caring for a person with a suspected haemorrhagic fever illness, a family member having a suspected haemorrhagic fever illness or touching a dead body of a person who was suspected to have died of a haemorrhagic fever illness.)
    - o Yes
    - o No

i. If yes, please state who you were in contact with (a relative, friend, patient), when this happened, what illness and where you were exposed (e.g. at home, in the hospital)?

.....

26. Have you been ill with **fever** within the last **3 weeks**?
    - o Yes, please state your **symptoms**

      .....
    - o No

i. **How many days after** the illness began did you seek first advice or treatment?

.....

ii. Where did you seek advice or treatment **first**?
    - o Village Health Team (VHT
    - o Private clinic
    - o Drug shop/Dispensary/Pharmacy
    - o Hospital/Health centre
    - o Herbalist/traditional healer
    - o Church
    - o Don't know
    - o Other, specify: .....

iii. Did you seek advice or treatment anywhere else during the whole course of your illness? (multiple answers)

- o Village Health Team (VHT
- o Private clinic
- o Drug shop/Dispensary/Pharmacy
- o Hospital/Health centre
- o Herbalist/traditional healer
- o Church
- o Don't know
- o Other, specify: .....

27. If you had a persistent fever (for 2 days or more), would you consider seeking advice or treatment outside your home?
- Yes
- No

i. **How many days after** an illness associated with fever began would you seek first advice or treatment?

.....

ii. Where would you most likely go to seek advice or treatment **first**?
- Village Health Team (VHT
- Private clinic
- Drug shop/Dispensary/Pharmacy
- Hospital/Health centre
- Herbalist/traditional healer
- Church
- Don't know
- Other, specify: .....

iii. Would you seek advice or treatment anywhere else during the whole course of your illness? (multiple answers)
- Village Health Team (VHT
- Private clinic
- Drug shop/Dispensary/Pharmacy
- Hospital/Health centre
- Herbalist/traditional healer
- Church
- Don't know
- Other, specify: .....

D. Questions about your animal contact.

28. Did you sleep under a **mosquito net** most nights of the past 12 months (past year)?
- Yes
- No

29. Have you noticed any sign of **rodents** in your house in the past 12 months (past year)?
- Yes
- No

30. Did any of these **insects bite** you in the past year? Tick all that apply (Have a look at the extra document with the pictures.)
- Mosquito
- Louse
- Housefly
- Mite
- Tick
- Flea
- None of the above

31. Have you **ever** been bitten by a **tick**, and if so, how often does this happen to you?
- Yes, daily
- Yes, weekly
- Yes, monthly
- Yes, few times a year
- Yes, once a year
- Yes, less than once year
- No

32. Have you **ever** been bitten by **mosquitoes**, and if so, how often does this happen to you?
- Yes, daily
- Yes, weekly
- Yes, monthly
- Yes, few times a year
- Yes, once a year
- Yes, less than once year
- No

AVP -

33. Have you seen **bats** roosting in your home, close to your home, or at your place of work in the past 12 months (past year)? o Yes o No	34. Have you has any direct contact with a bat in the past 12 months (past year)? o Yes o No	

35. Do you participate in regular activities that involve caring for animals at home or at work? (Includes milking, taking the animal for grazing, spraying or cleaning their sleeping place)
- o Yes, daily
- o Yes, weekly
- o Yes, monthly
- o Yes, few times a year
- o Yes, once a year
- o Yes, less than once year
- o No

i. Which activities have you carried out while caring for animals? (tick all that apply)
- o Milking
- o Taking the animal for grazing
- o Spraying with acaricides
- o Cleaning animal dung
- o Cleaning their sleeping place
- o Delivery of offspring (lambs, calves)
- o Washing the animal
- o Dhorning
- o Castration
- o None
- o Other, specify: .....

36. Do you **keep** any of these **animals**? (tick all that apply)
- o Cows
- o Goats
- o Sheep
- o Pigs
- o Poultry
- o Dog
- o Cat
- o Guineapig
- o Other, specify .....
- o No animals

i. Do you consider any of them as a **pets**? They stay with you indoors and/or are cuddled by you and your family
- o Yes
- o No

ii. If yes, which animal(s)?
- o Cows
- o Goats
- o Sheep
- o Pigs
- o Poultry
- o Dog
- o Cat
- o Guineapig
- o Other, specify .....

iii. Are there times that **animals sleep inside house**? (e.g. due to fear of thieves, during rain or cold nights)

- Yes, daily
- Yes, weekly
- Yes, monthly
- Yes, few times a year
- Yes, once a year
- Yes, less than once a year
- No

iv. If yes, which animal(s)?

- Cows
- Goats
- Sheep
- Pigs
- Poultry
- Dog
- Cat
- Guineapig
- Other, specify .....

v. What do you do if you see **ticks on your animals**? (tick all that apply)

-
- I don't see ticks on my animals
- Spray with acaricide
- Pick off with hands (without gloves)
- Pick off with an instrument or with gloves on
- Pierce
- Apply ghee/jelly/paraffin
- Nothing
- Other, specify .....

vi. Have any of the animals you care for **died** suddenly in the past 12 months (past year)? (This includes abortions in pregnant animals.)

- Yes, please tell us any details about the illness the animal had e.g. breathing difficulty, injury, bleeding etc.

  .....
- No

vii. If yes, which animal(s)?

- Cows
- Goats
- Sheep
- Pigs
- Poultry
- Dog
- Cat
- Guineapig
- Other, specify: .....

viii. After an animal died, what did you do with the animal?

- Prepared and eaten
- Sold for human consumption
- Sold for animal consumption
- Buried
- Fed to pigs
- Fed to dogs
- Burned
- Other, specify: .....

37. Have you hunted, touched, or eaten a **wild bird** in the past 12 months (past year)?
- o Yes, daily
- o Yes, weekly
- o Yes, monthly
- o Yes, few times a year
- o Yes, once a year
- o Yes, less than once a year
- o No

i. Which type of bird did you have contact with?

.....

ii. Which type of contact did you have with the animal(s)?
- o Hunted the bird
- o Killed the bird
- o Ate the bird
- o Pecked/bitten by the bird
- o Picked up the bird
- o Petted the bird
- o Other, specify: .....

iii. How did you hunt and/or kill the bird(s)?
- o With a knife or machete
- o With a gun
- o With your hands or feet
- o Using a trap
- o Other, specify: .....

38. Have you hunted, touched, or eaten a **wild animal** in the past 12 months (past year)?
- o Yes, daily
- o Yes, weekly
- o Yes, monthly
- o Yes, few times a year
- o Yes, once a year
- o Yes, less than once a year
- o No

i. What type of animal(s) did you have contact with?
- o Antelope
- o Buffalo
- o Ugandan Kop
- o Warthog
- o Edible rat
- o Rodent
- o Bird
- o Stray dog
- o Other, specify: .....

ii. Which type of contact did you have with the animal(s)?
- o Hunted the animal
- o Killed the animal
- o Ate the animal
- o Was bitten by the animal
- o Picked up the animal
- o Petted the animal
- o Other, specify: .....

iii. How did you hunt and/or kill the animal?
- o With a knife or machete
- o With a gun
- o With your hands or feet
- o Using a trap
- o Other, specify: .....

39. Have you ever **picked (collected) ticks** from animals?

- Yes, daily
- Yes, weekly
- Yes, monthly
- Yes, few times a year
- Yes, once a year
- Yes, less than once a year
- No

i. If yes, how have you **discarded** them?

- Crushed with hands
- Crushed with stone
- Burned
- Thrown away
- Buried
- Other, specify .....

40. Have you ever eaten ticks?

- Yes, raw
- Yes, roasted
- No

41. Have you **slaughtered** an animal over the past 12 months (past year)?

- Yes, 1 – 10 animals
- Yes, 11 – 30 animals
- Yes, more than 30 animals
- No

i. If yes, please specify which animal(s)

- Cattle
- Goat
- Sheep
- Pig
- Poultry
- Other, specify: .....

ii. While slaughtering animals have you ever been aware of an open wound (a cut or scrape) on your hands or arms?

- Yes
- No

iii. When you slaughter animals, do you usually use personal protective equipment (gloves, apron, boots)?

- Yes, always
- Yes, sometimes
- No

iv. If yes, specify the personal protective equipment that you use.

- Gloves
- Rubber boots
- Protective googles
- Apron
- Mask

42. Have you participated in the **slaughter** of an animal over the past 12 months (past year)?
(eg stood close-by or restrained the animal)
- Yes, 1 – 10 animals
- Yes, 11 – 30 animals
- Yes, more than 30 animals
- No

i. If yes, please specify which animal(s) were slaugthered
- Cattle
- Goat
- Sheep
- Pig
- Poultry
- Other, specify: .....

ii. While participating in slaughtering animals, have you ever been aware of an open wound (a cut or scrape) on your hands or arms?
- Yes
- No

iii. When you participate in the slaughter animals, do you usually use personal protective equipment (gloves, apron, boots)?
- Yes, always
- Yes, sometimes
- No

iv. If yes, specify the personal protective equipment that you use.
- Gloves
- Rubber boots
- Protective googles
- Apron
- Mask

43. Have you **skinned** an animal over the past 12 months (past year)?
- Yes, 1 – 10 animals
- Yes, 11 – 30 animals
- Yes, more than 30 animals
- No

i. If yes, please specify which animal(s)
- Cattle
- Goat
- Sheep
- Pig
- Poultry
- Other, specify: .....

ii. Have you been aware of an open wound (a graze or scrape) on your hands or arms while skinning animals?
- Yes
- No

iii. Did you use personal protective equipment (gloves, apron, boots) while skinning animals?
- Yes, always
- Yes, sometimes
- No

iv. If yes, specify the personal protective equipment that you used.
- Gloves
- Rubber boots
- Protective googles
- Apron
- Mask

44. Have you **handled or processed fresh animal skin** over the past 12 months (past year)?
- Yes, 1 – 10 skins
- Yes, 11 – 30 skins
- Yes, more than 30 skins
- No

i. If yes, please specify from which animal(s)
- Cattle
- Goat
- Sheep
- Pig
- Poultry
- Other, specify: .....

ii. Have you been aware of an open wound (a graze or scrape) on your hands or arms while processing animal skin?
- Yes
- No

iii. Did you use personal protective equipment (gloves, apron, boots) while processing animal skin?
- Yes, always
- Yes, sometimes
- No

iv. If yes, specify the personal protective equipment that you used.
- Gloves
- Rubber boots
- Protective googles
- Apron
- Mask

45. Have you **butchered** an animal over the past 12 months (past year)?
- Yes, 1 – 10 animals
- Yes, 11 – 30 animals
- Yes, more than 30 animals
- No

i. If yes, please specify which animal(s)
- Cattle
- Goat
- Sheep
- Pig
- Poultry
- Other, specify: .....

ii. When butchering an animal, have you been aware of an open wound (scratch or graze) on your hands or arms?
- Yes
- No

iii. When butchering animals, do you use personal protective equipment (gloves, apron, boots)?
- Yes, always
- Yes, sometimes
- No

iv. If yes, specify the personal protective equipment that you use
- Gloves
- Rubber boots
- Protective googles
- Apron
- Mask1

46. Are there any additional comments that you would like to make?
Please note any problems during the interview here:

.....
.....
.....

Thank you for your time! We will now collect your blood sample and provide your financial compensation.

E. Sample collection

47. Sample collected by .....
48. Specimen taken:
    - o Serum
    - o None
49. Date serum sample taken .....
50. Volume of blood taken in serum tube (mL) .....
51. Additional notes

.....
.....

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