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Crimean-Congo Haemorrhagic Fever in Kenya

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ABSTRACT

Crimean Congo haemorrhagic fever (CCHF) is an emerging, tick-borne viral pathogen. Found on three continents, it is the most widespread of all tick-borne pathogens, but accurate geographical limits and epidemiology in Africa are still mostly unknown. Ticks act as both vectors and reservoirs, and the transmission cycle involves both wild and domestic animals and may occasionally spill over to humans. Further healthcare-related infections from human to human are common. With a high mortality rate and no cure or vaccine, CCHF is considered a major public health threat in endemic countries.

This licentiate thesis consists of a literature review and an experimental work section. The literature review covers the basics of tick ecology, tick-borne diseases and viral haemorrhagic fevers with a focus on Africa and Kenya. These are used as foundations to understand CCHF in detail, encompassing virology, epidemiology, diagnostics, symptoms, treatment and prevention. The experimental work entails PCR-screening of ticks collected from South-eastern Kenya for the CCHF virus.

The main objective of the study was to find whether CCHF is circulating in free-roaming ticks collected from two conservancies in the Taita Hills area. Taita Hills are located in Taita-Taveta county, near the Helsinki University research centre in Wundanyi. The ticks were collected by the Vapalahti virology team in 2018. This thesis involved the RNA extraction and measurement from the ticks and screening for CCHF virus with RT-qPCR. The results were negative for all 57 units of ticks processed. The study was a part of a larger research project, “Preparedness for emerging zoonotic infections in Kenya”.

Previous publications on CCHF are lacking from this part of Kenya, so this study was a valuable part of primary research to establish the geographical limits and members of the enzootic cycle in Taita Hills. It would be essential to continue examining ticks from animal sources in addition to human serology, to further establish evidence of possible CCHF occurrence in the area. Mapping the prevalence and epidemiology of zoonotic and tick-borne pathogens is especially critical now, when climate change and diminishing biodiversity stir and alter disease emergence in an unprecedented manner.

Keywords
Crimean-Congo haemorrhagic fever virus, viral haemorrhagic fever, tick, tick-borne, arbovirus, zoonosis, public health

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TIIVISTELMÄ

Krimin-Kongon verenvuotokuume on puutiaisvälitteinen virustauti. Sen kolme manteretta kattava levinneisyysalue tekee siitä maantieteellisesti kaikkein laaja-alaisimman punkkivälitteisen sairauden. Tarkat levinneisyysrajat ja epidemiologia Afrikassa ovat silti pääosin hämärän peitossa. Punkit toimivat sekä vektoreina että viruksen säilymöinä. Taudin luontainen kiertokulku sisältää sekä villi- että kotieläimiä ja voi joskus tarttua myös ihmiseen. Lisäksi ihmisten jatkotartunnat terveydenhuollossa ovat yleisiä. Tautia pidetään merkittävänä kansanterveydellisenä uhkana endeemisissä maissa, koska sillä on korkea kuolleisuus eikä siihen ole hoitoa tai rokotetta.

Tässä lisensiaattityössä on kirjallisuuskatsaus ja tutkimusosuus. Kirjallisuuskatsauksessa perehdytään punkkien ekologiaan, punkkivälitteisiin tauteihin ja verenvuotokuumeisiin, painottuen Afrikkaan ja Keniaan. Näitä käytetään taustatietona Krimin-Kongon verenvuotokuumeen syvälliseen ymmärtämiseen, sisältäen taudin virologian, epidemiologian, diagnostiikan, oireet, hoitomahdollisuudet ja ehkäisyn. Tutkimusosuus käsittelee kaakkoisesta Keniasta kerättyjen punkkien PCR-tutkimusta viruksen varalta.

Tutkimuksen päätavoite oli selvittää, löytyykö Krimin-Kongon verenvuotokuumevirusta maastosta kerätyistä punkeista Taitavuorten alueelta. Taitavuoret sijaitsevat Taita-Tavetan piirikunnassa, lähellä Helsingin yliopiston tutkimusasemaa Wundanyin kaupungissa. Punkit keräsi prof. Vapalahden virologian tutkimusryhmä vuonna 2018. Tähän lisensiaattityöhön kuului RNA:n eristys ja mittaaminen punkeista sekä viruksen etsiminen RT-qPCR-tekniikalla. Tulokset olivat negatiivisia kaikkien 57:n punkkiyksikön kohdalla.

Aikaisempia julkaisuja Krimin-Kongon verenvuotokuumeeseen liittyen tältä alueelta ei ole, joten tämä tutkimus oli tärkeä osa levinneisyyden ja taudin kiertokulun selvittämistyötä. Olisi tärkeää jatkaa myös eläimistä irrotettujen punkkien sekä ihmisten vasta-aineiden tutkimista, jotta saataisiin lisätodisteita siitä, esiintyykö alueella Krimin-Kongon verenvuotokuumetta. Zoonoottisten ja punkkivälitteisten tautien selvitystyö on erityisen tärkeää nyt, kun ilmastonmuutos etenee samalla luonnon monimuotoisuuden köyhtyessä aiheuttaen ennennäkemättömiä muutoksia tautien esiintymisessä.

Avainsanat
Krimin-Kongon verenvuotokuume, verenvuotokuume, punkki, punkkivälitteinen, arbovirus, zoonoosi, kansanterveys

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1 Introduction

The African continent supports many zoonotic diseases, of which the most notorious is most likely the Ebola virus. A lesser-known fact is that among the group of viral haemorrhagic fevers there is another illness, known for a thousand years and causing deadly epidemics in Africa, Europe and Asia. This licentiate thesis examines Crimean-Congo haemorrhagic fever (abbreviated CCHF), an emerging tick-borne disease with a high fatality rate and no cure. Due to its nature, farmers, butcherers, veterinarians and healthcare workers are among the risk groups. Other coexisting febrile illnesses as well as lacking laboratory resources can often cause confusion in the diagnostic process, causing further infections. Information on the geographical limits and epidemiology of CCHF in Africa is inconsistent paired with very poor surveillance systems in place, if any. Climate change and diminishing biodiversity may affect its boundaries in unknown and rapid ways. According to the World Health Organisation, CCHF has "potential to cause a public health emergency" and is in "urgent need for accelerated research and development".

My objective was to deepen my knowledge on ticks, viral haemorrhagic fevers and the zoonotic disease burden in Africa and Kenya. Using these topics as background I aimed to better understand the significance of CCHF in Kenya and worldwide. The experimental work aspired to answer whether CCHF virus circulates in ticks collected from two conservancies close to the Helsinki University research centre in Taita Hills, as part of a larger project known as "Preparedness for emerging zoonotic infections in Kenya". Broader themes related to this project are explored throughout the work.

The literature review proceeds from tick-borne diseases in general to viral haemorrhagic fevers and CCHF. First, the review introduces Kenyan ticks, their ecology and the tick-borne diseases they carry. Secondly, viral haemorrhagic fevers are presented before specifically delving into CCHF. The paper conveys a thorough image of the disease including history, microbiology, epidemiology, clinical signs, treatment, diagnostics and prevention. Finally, the experimental work and conclusions drawn from the results and literature are presented in the last part.

A - LITERATURE REVIEW

2 Tick vectors and tick-borne diseases in Kenya

2.1 Country details

Kenya is situated on the Equator in East Africa. It is bordered by 5 countries, Lake Victoria and a 500-kilometre stretch of the Indian Ocean. Due to the exceptional geography of this 580 000 km^2 country, it hosts climates from tropical to arid and vegetation ranging from deserts to savannahs and rainforests and is considered one of the world's 25 biodiversity hotspots (Beck et al. 2018, Myers et al. 2000). Kenya is also home to a variety of wildlife species and a production animal population of over 200 million (OIE WAHIS Interface 2018). Roughly two thirds of the 50+ million inhabitants are farmers or derive their livelihoods indirectly from agriculture (United Nations 2019, KNBS 2016). Consequentially, the country is also a hotspot for various tick species and thus well suited for observations of parasite-host interactions, in humans and animals alike (Keesing et al. 2013).

2.2 Ixodid tick species and their ecology

Ticks are blood-feeding ectoparasites and have their own order in the phylum Arthropoda, consisting of two major families *Ixodidae* and *Argasidae*, which are commonly called hard ticks and soft ticks, respectively (Roe & Sonenshine 2014). The focus of this work is the ixodid group, of which the largest and most important genera are *Ixodes*, *Dermacentor, Haemaphysalis*, *Rhipicephalus* (subgenus *Boophilus*), *Hyalomma* and *Amblyomma* (Deplazes et al. 2016). Four of these genera can be found in Kenya, where *Hyalomma* and *Rhipicephalus* are the most numerous in species and have the greatest veterinary importance. The genera *Haemaphysalis* and *Amblyomma* are less prominent in number and as disease vectors but each include single species of high veterinary interest (Madder et al. 2013, Walker et al. 2003).

All ixodid ticks follow a life cycle of four stages: egg, larva, nymph and adult (Apanaskevich & Oliver 2014). A varying number of blood meals is required for transition (also known as moulting) from one stage to another, except for hatching from egg to larvae (Deplazes et al. 2016). The number of hosts the ticks feed on during their life cycle divides them into 1-, 2- or 3-host ticks, and the host number may differ inside a genus. An obligate 3-host cycle is most

common for *Haemaphysalis*, *Rhipicephalus* and *Amblyomma*. Some species, such as certain *Hyalomma* ticks, are able to modify their life cycle (e.g. number of hosts) according to their environment (Apanaskevich & Oliver 2014). Ticks that utilise a broad variety of host species are classed as euroxenous whilst stenoxous ticks only have a few preferential hosts (Deplazes et al. 2016). The preferred host is also dependent on the tick's stage in its life cycle (Apanaskevich & Oliver 2014). Generally, most ixodid ticks in Kenya infest both domestic and wild mammals as well as birds. For adult ticks, the host array is composed mainly of ungulates and carnivores, while larvae and nymphs usually feast on smaller animals such as leporids and rodents. Ticks that infest snakes, lizards and tortoises mainly belong to the genus *Amblyomma* (Madder et al. 2013, Walker et al. 2003).

Ticks can be further categorised by their manner of host location, known as questing (Apanaskevich & Oliver 2014). Nidicolous or endophilic ticks burrow into their host's nests and latch onto them there. Pasture ticks, such as many *Haemaphysalis* and *Rhipicephalus*, quest on their prey by climbing on vegetation elevated from the ground, in order to latch onto bypassing animals. The hunters, as their name suggests, actively follow their targets. This is a tactic employed by *Amblyomma* and *Hyalomma*. Both ambushing and hunting behaviours that take place in open terrain are considered exophilic or non-nidicolous (Apanaskevich & Oliver 2014, Walker et al. 2003, Madder et al. 2013). Distinctions between questing methods are noteworthy in tick sample collection: ambushing ticks can be collected from vegetation with special host-mimicking traps whereas the hunters are most conveniently harvested from host animals. Nidicolous ticks are best trapped in their preferred host's nests (Walker et al. 2003). Classification into endophilic and exophilic is not always straightforward. *Hyalomma* spp., for instance, exhibit some ambivalence in their questing patterns, a characteristic which probably originates from the harsh environments they inhabit. They may combine both active hunting and burrowing into stables and other animal shelters to wait for their hosts (Apanaskevich & Oliver 2014).

2.3 Tick-borne diseases

Ticks cause disease by acting as vectors for pathogens or by causing primary irritation, inflammation and immunological responses (Deplazes et al. 2016, Wikel 2014). Besides their ability to cause primary tick disease, the most important role of pathogen transmission is played by ixodid ticks (Roe & Sonenshine 2014). Significant viral zoonoses transmitted by hard ticks in

Kenya are Crimean-Congo haemorrhagic fever and Nairobi sheep disease (Sang et al. 2011, Lwande et al. 2012, Krasteva et al. 2020). Protozoal pathogens include the genera *Babesia* and *Theileria* (Adjou Moumouni et al. 2015). Important intracellular bacterial infections involve *Rickettsia* spp., *Ehrlichia* spp., *Anaplasma* spp. and *Coxiella burnetii* (also known as Q-fever) (Knobel et al. 2013, Koka et al. 2017, Ringo et al. 2019). Some of the most common ixodid tick species of veterinary importance and the specific diseases they may transmit in Kenya are assembled in Table 1. Compared to mosquitoes, ticks infect less individuals with vector-borne diseases, but with a broader variety of pathogens including bacteria, viruses, protozoa, nematodes and even fungi. A multitude of these diseases are also zoonoses (Roe & Sonenshine 2014, Deplazes et al. 2016).

Conditions caused by argasid ticks in Kenya are predominantly primary skin lesions and sometimes toxicoses, with a few important exceptions, such as African swine fever virus and relapsing fever (causative bacterium *Borrelia duttoni*) transmitted by species in the *Ornithodoros*-genus (Walker et al. 2003). As opposed to ixodids, argasids rarely induce paralysis in host animals, possibly due to their considerably shorter feeding times (Estrada-Peña & Mans 2014).

Tick infestation causes mechanical injury and triggers multiple defence mechanisms in the body, which may result in local lesions but also systemic effects. Tick-saliva and other molecules related to attachment cause immunosuppression and predisposes animals to diseases they might not otherwise acquire (Deplazes et al. 2016, Wikel 2014). Locally the attachment site may get irritated or even necrotic. If the infestation is numerous, the area may be prone to secondary infections, such as dermatophilosis or abscess formation (Walker et al. 2003). Blood loss and consequent anaemia may also occur in these situations (Roe & Sonenshine 2014). In addition, wounded areas are susceptible to myiasis or flystrike, an infestation of fly larvae in the skin, which is potentially lethal when the animal is heavily affected (Deplazes et al. 2016).

Systemic toxicoses are complications of tick infestations for domestic animals in warm climates (Walker et al. 2003). Non-specific toxicosis, commonly known as tick worry, can occur after heavy tick infestation and is related to an immunological reaction of the host. Cattle may become irritable and restless and subsequently lose condition (Deplazes et al. 2016). Specific toxicosis, of which two conditions called sweating sickness and tick paralysis are known, is caused by neurotoxins excreted in tick saliva (Estrada-Peña & Mans 2014, Walker et al. 2003).

Table 1. Some important tick species of veterinary interest in Kenya and the diseases they transmit according to Madder et al. (2013), Walker et al. (2003) and Knobel et al. (2013).

Tick species	*Preferred main mammalian host*	*Disease vectorship*
Amblyomma variegatum	Wild and domestic ungulates, such as cattle, buffaloes and giraffes, warthogs, rhinoceros (black/white)	*Ehrlichia ruminantium* (heartwater), *E. bovis* (bovine ehrlichiosis), *Theileria mutans*, *T. velifera* (bovine theileriosis), Nairobi sheep disease virus, *Dermatophilus congolensis* (dermatophilosis)
A. lepidum	Cattle, camels, various other livestock animals, wild ungulates	*Ehrlichia ruminantium*, *Theileria mutans*, *T. velifera*
Haemaphysalis leachi	Domestic dogs and wild carnivores	*Babesia canis*, *Coxiella burnetii* (Q-fever), *Rickettsia conorii* (human tick typhus)
Hyalomma dromedarii	Camels, cattle, sheep, goats, horses	*Theileria annulata* (tropical theileriosis; mechanical vector of the camelpox virus)
Hy. rufipes	Ruminants, horses, large wild herbivores	*Anaplasma marginale* (bovine anaplasmosis), *Babesia occultans* (bovine babesiosis), Crimean-Congo Haemorrhagic Fever, *Rickettsia conorii*
Hy. truncatum	Cattle, sheep, goats, horses, large wild herbivores, occasionally dogs	Crimean-Congo Haemorrhagic Fever, *Rickettsia conorii* Toxicosis (sweating sickness)
Rhipicephalus decoloratus	Cattle, wild antelopes, horses, zebras	*Babesia bigemina*, *Anaplasma marginale*, *Borrelia theileri* (spirochaetosis)
R. appendiculatus	Cattle, goats, African buffalo, wild antelopes	*Theileria parva* (East Coast Fever), *Theileria taurotragi* (bovine theileriosis), *E. bovis*, Nairobi sheep disease virus, *Rickettsia conorii*
R. sanguineus	Dogs	*Ehrlichia canis*
R. evertsi evertsi	Horses, zebras, antelopes, cattle, sheep	*Theileria equi*, *Babesia caballi* (equine piroplasmosis), *B. bigemina*, *Theileria separata*, *Babesia theileri*
R. pulchellus	Cattle, sheep, goats, zebras, camels, African buffaloes, antelopes	Nairobi sheep disease virus
R. praetextatus	Cattle, dogs, camels, wild carnivores, warthogs, zebras	Nairobi sheep disease virus

Sweating sickness usually impacts calves, which develop an acute dermatitis accompanied by fever. Tick paralysis affects both animals and humans. It begins as a weakness or incoordination (ataxia) of the limbs and may progress into complete paralysis, respiratory failure and death. The process may be reversed by a timely removal of ticks (Estrada-Peña & Mans 2014). Furthermore, tick-related diseases cause financial losses in domestic animals by way of reduced welfare, increased mortality, decreased production (milk, meat) and lower quality leather resulting from skin damage (Deplazes et al. 2016).

3 Viral haemorrhagic fevers

Viral haemorrhagic fevers (VHF) are a group of diseases from four families of RNA-viruses causing a clinically similar syndrome (Paessler & Walker 2013). They are found almost everywhere on the globe, yet are usually tied to their preferred natural cycles, which may involve domestic and wild animals, humans, mosquitoes and ticks (Blumberg et al. 2014). Currently, Africa hosts 8 known haemorrhagic fever viruses (Table 2). Although heterogeneous in their ecology and pathophysiology, they share some common clinical signs which culminate into a severe febrile illness with cardiovascular damage (Paessler & Walker 2013). Initial symptoms often include fatigue, nausea and myalgia. Later, gastrointestinal and central nervous system involvement may be seen. Haemorrhages in various locations are possible but not present in all cases and are rarely the main cause of death (Peters & Zaki 2011). Rather, the life-threatening events in fatal cases are often the processes related to systemic inflammatory response syndrome (SIRS), such as cytokine storm and complement activation, leading up to multiple organ failure. In addition, fatal coagulation disturbances may originate from the typical hepatic necrosis causing insufficiency in the production of coagulation factors, which are simultaneously used in excess in a process of disseminated intravascular coagulation (DIC) (Paessler & Walker 2013). The actual involvement of the vascular component is thus not limited to excess bleeding but comprises of an overall disturbance and collapse of the haemostatic system (Blumberg et al. 2014).

In some VHFs only a small portion of patients develop a significant disease, and the illness may go unnoticed; in some the fatality rate can be as high as 80% (Blumberg et al. 2014). Usually only humans become afflicted, the animal reservoirs excluding non-human primates in the sylvatic cycle remain unaffected. Controlling and preventing haemorrhagic fevers is difficult due to their zoonotic and often arboviral nature and most of them have neither approved vaccines nor valid treatment options other than supportive care (Paessler & Walker 2013). Pest control targeting mainly ticks, mosquitoes and rodents alongside with standard barrier nursing routines and hygiene practices concerning animals and agricultural practices form the basis of VHF prevention. Diagnostics are complicated by indistinguishable general symptoms, similar endemic diseases as differentials or coinfections and non-infectious syndromes. Access to diagnostic tools may also be very limited in resource-poor areas and conflicts often interfere with epidemiological investigations (Blumberg et al. 2014).

Table 2. Significant viral haemorrhagic fevers affecting humans in Africa (Paessler & Walker 2013, Blumberg et al. 2014, Peters & Zaki 2011).

Family	*Disease*	*Geography*	*Epidemiology*
Arenaviridae	Lassa fever	West Africa	Vector/reservoir: *Mastomys* spp. rodents Spreads from rodents to humans and between humans
	Lujo	Zambia	Vector/reservoir: unknown, rodents suspected Single outbreak of five human cases
Bunyaviridae	Crimean-Congo Haemorrhagic Fever	Worldwide (Europe, Asia, Africa)	Vector/reservoir: ticks, *Hyalomma* spp. in particular, circulates in vertebrates via tick bite Human infection from domestic animals and tick bites and between humans
	Rift Valley Fever	Sub-Saharan Africa	Vector/reservoir: *Aedes* spp.-mosquitoes Primarily an animal pathogen; human infection from mosquito bites and infected animals
Filoviridae	Ebola*	Central and Equatorial Africa, (Philippines**)	Vector/reservoir: unknown, bats suspected Sporadic human outbreaks often include infected nonhuman primates, transmission between humans through close contact
	Marburg	Central and Equatorial Africa	Vector/reservoir: fruit bats Sporadic human outbreaks often include infected nonhuman primates, transmission between humans through close contact
Flaviviridae	Dengue	Tropics, subtropics	Vector/reservoir: *Aedes* spp.-mosquitoes Human infection via mosquito bites
	Yellow fever	Africa	Vector/reservoir: *Aedes* spp.-mosquitoes, sylvatic cycle includes nonhuman primates Human infection via mosquito bites

*Currently divided into 6 subtypes with varying pathogenicity and geographical origin: Zaïre, Sudan, Reston, Taï Forest (formerly Côte d'Ivoire), Bundibugyo, Bombali. **Ebola Reston, asymptomatic infection.

4 Crimean-Congo haemorrhagic fever

4.1 General description

Crimean-Congo haemorrhagic fever (CCHF) is an emerging viral haemorrhagic fever of growing interest with a geographical distribution on three continents (Bente et al. 2013, Al-Abri et al. 2017). CCHF is a tick-borne virus spread by numerous ixodid ticks, especially in the genus *Hyalomma* (Hoogstraal 1979). Wild and domestic animals act as its reservoir without falling ill, while humans may experience a deadly disease (Bente et al. 2013). The enzootic cycle of CCHF between ticks and vertebrates encompasses a variety of animal species, including small mammals, equids, camelids and bovids (Spengler et al. 2016). Only one report of reptilian antibodies has been recorded even with the presence of suitable reptile-infesting vectors (Whitehouse 2004). Birds (excluding ostriches) seem to be refractory to the disease and rarely present viremia (Spengler et al. 2016). Nevertheless, birds are thought to have an important but not yet fully understood role in the epidemiology of CCHF (Al-Abri et al. 2017, Spengler et al. 2016). Migratory birds from Africa with CCHFV-positive tick infestations have been found in several European countries (Gale et al. 2012, Leblebicioglu et al. 2014, De Liberato et al. 2018). Other wild animal populations and their contacts with domestic animals may also present possibilities for CCHF transmittance and shifts in endemicity, such as large ruminant migrations (Lwande et al. 2012).

Farm and animal workers, butchers and veterinary staff along with healthcare workers are the main risk populations for human CCHF seropositivity and clinical disease (Nasirian 2019). The level of medical care and surveillance also affects CCHF incidence and prevalence, sometimes leading to more infections being reported from areas where CCHF circulation is lower and less reports being made from high risk locations (Messina et al. 2015, Al-Abri et al. 2017, Ftika & Maltezou 2012). Various social changes and conflicts may spur CCHF circulation, as observed in the first recorded epidemic in Crimea during the 2nd World War and later in the Kosovo War (Sargianou & Papa 2013, Esser et al. 2019). Several other social and cultural aspects increase the risk of contracting CCHF, such as ritual sacrifice of domestic animals in religious festivals, a practice common in the geographical distribution areas of CCHF (Sargianou & Papa 2013, Leblebicioglu et al. 2015).

In comparison to other VHF's, CCHF is highly transmissible from human to human and is characterized by especially severe haemorrhagic symptoms, early DIC and hepatic involvement (Blumberg et al. 2014). Due to its contagiousness, limited treatment options leading to relatively high fatality rates in humans and need for immediate further research, the World Health Organisation classified CCHF as a priority viral pathogen in 2018, along with four other haemorrhagic fevers (WHO 2018). The disease is also listed in the "multiple species diseases, infections and infestations" -category of the World Organisation for Animal Health (OiE), which makes the virus notifiable and has international trade implications, amongst other effects (OiE 2019).

4.2 History

A disease fitting the description of Crimean-Congo haemorrhagic fever has been recognised in Central Asia for almost a millennium, including depictions of the parasite believed to spread it. In Uzbekistan, the haemorrhagic disease was known as "black death" long before the plague pandemic in the Middle Ages (Whitehouse 2004). The pathogen was rediscovered by modern medicine during the Crimean offensive in 1944, when Soviet military troops in the Crimea region fell ill in an outbreak of the disease (Hoogstraal 1979). This sparked research in the Soviet Union finally leading up to the confirmation of the pathogen being a viral agent transmitted by ticks during the next two decades. Simultaneously the "Congo virus" was detected from Belgian Congo, Uganda and Nigeria (Bente et al. 2013). Accompanied by many separate haemorrhagic fever viruses from Central Asia and Africa, the Congo virus was ultimately shown to be the same pathogen as the one causing the Crimean epidemic. Hence the name Crimean-Congo haemorrhagic fever was born, representing the near-worldwide spread of this versatile pathogen (Whitehouse 2004).

4.3 Virology

4.3.1 Taxonomy

Crimean-Congo haemorrhagic fever virus (CCHFV) is a negative-sense, single-stranded RNA virus belonging to the order *Bunyavirales*, family *Nairoviridae*, and the genus *Orthonairovirus*. Currently the family consists of three genera: *Orthonairovirus*, *Shaspivirus* and *Striwavirus*. Orthonairoviruses are further divided into 7 serogroups, totalling 15 species of viruses (ICTV 2019).

Besides CCHF, the only other significant pathogen in the genus is the Nairobi sheep disease virus, which is the causative agent of a high-mortality haemorrhagic gastroenteritis disease mainly seen in sheep and goats. Nairobi sheep disease, as the name suggests, was first reported in Nairobi and circulates endemically in East Africa (Krasteva et al. 2020). The virus also has an Asian variant known as the Ganjam virus (Yadav et al. 2011). In addition to Nairobi sheep disease virus, the non-pathogenic Hazara virus of the CCHF serogroup is thought to be a promising model for future antiviral agents and vaccines for CCHF (Dowall et al. 2012, Krasteva et al. 2020).

4.3.2 Virion structure

Morphologically the CCHF virion is spherical with a diameter of 90-100 nm and studded with two types of glycoproteins on the host cell derived bilayer lipid envelope (Bergeron et al. 2007, Lasecka & Baron 2014). As with all nairoviruses, the genome is divided into three segments, S, M and L, each enclosed by nucleocapsid proteins (N) and the RNA-dependent RNA polymerase (RdRp or L-protein). The genome segments are responsible for coding the nucleocapsid proteins, glycoproteins and RNA-polymerase, respectively (Lasecka & Baron 2014). The simplified structure of the virion is presented in Figure 1.

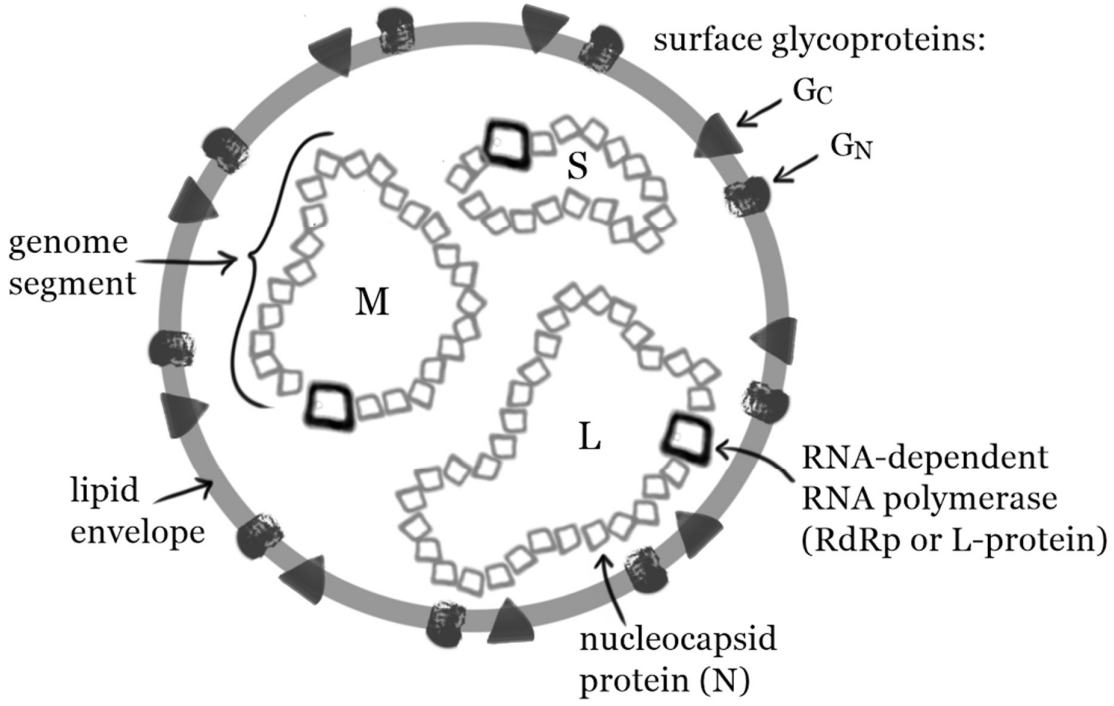

Figure 1. Schematic illustration of the CCHF virion.

4.3.3 Replication

The surface glycoproteins G_N and G_C are responsible for binding to cellular receptors of the host cells and for triggering neutralizing antibody reactions of the host. The precise cellular receptor in the host cells is yet to be identified (Zivcec et al. 2016). After internalisation by means of clathrin-dependent endocytosis, the viral envelope and the host endosome fuse to release the nucleocapsids and their genomic content (Garrison et al. 2013). The RNA-dependent RNA-polymerase generates messenger and complementary RNA for translation into nucleoproteins and genome replication (Zivcec et al. 2016). Due to the two types of glycoproteins, the messenger RNA of the M-segment is forwarded to the endoplasmic reticulum for translation and G_N/G_C precursor cleavage (Bertolotti-Ciarlet et al. 2005). Further processing of the glycoproteins is completed in the Golgi complex, alongside with the final virion assembly from the newly formed nucleocapsids (Bergeron et al. 2007). Finally, the virions are expelled from the host cell via exocytosis usually without notable cell damage (Zivcec et al. 2016).

4.4 Epidemiology

4.4.1 Geographical distribution

Crimean-Congo haemorrhagic fever is distributed throughout vast areas of Europe, Asia and Africa, making it the most widespread of all tick-borne pathogens, as represented in Figure 2 (Messina et al. 2015, Bente et al. 2013). The worldwide dispersal of the disease closely follows the distribution of the main vector, *Hyalomma* spp. ticks, the 50º Northern latitude being their northernmost limit of distribution (Al-Abri et al. 2017, WHO 2017). Scientific evaluation is challenging due to varying surveillance systems of CCHF and a lack of published literature even when cases are known to have been reported to authorities (Bente et al. 2013, Al-Abri et al. 2017). Precise geographical borders are therefore problematic to draw, in addition to constant migration and emergence of the virus (Spengler et al. 2019, Mild et al. 2010). The most accurate distribution maps are most likely created via extensive meta-analysis and layering of different factors, as done in the work of Messina et al. (2015), where a number of ecological variables were linked with serological, virological and clinical data. It was also noted that many countries with a high-risk profile lacked sufficient publications and reports of CCHF (Messina et al. 2015).

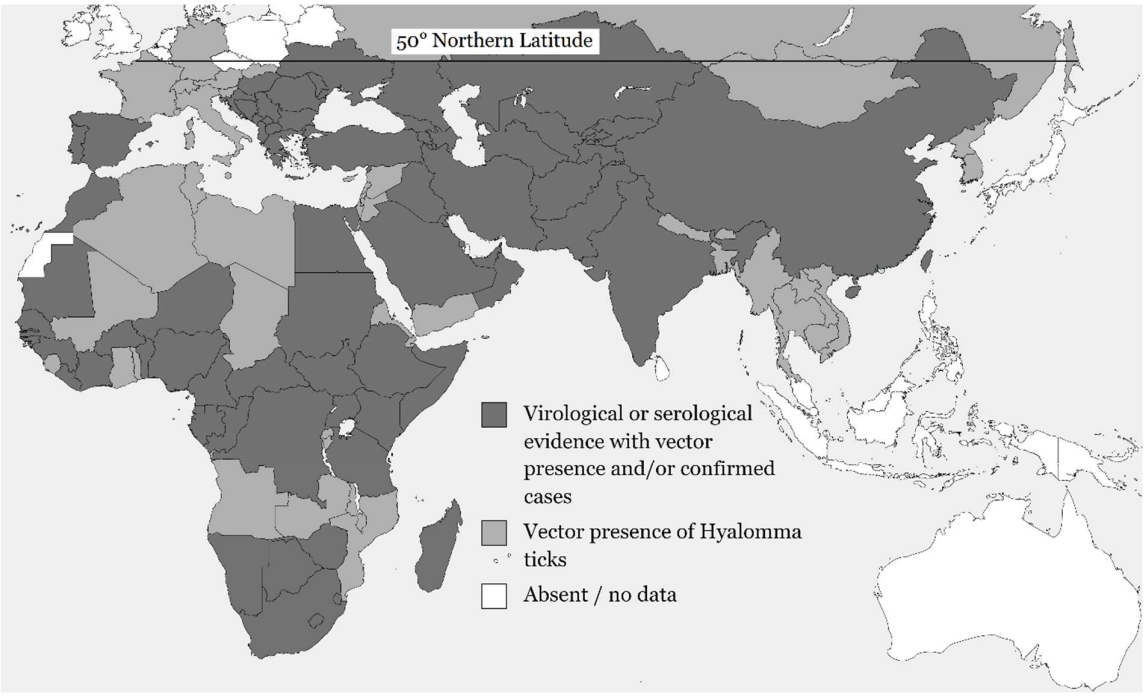

Figure 2. The geographical distribution of CCHF according to the World Health Organisation (2017).

In Kenya, antibodies have been detected from various parts of the country and from both rural and urban populations as well as domestic and wild animals (Lwande et al. 2012, Tigoi et al. 2015, Morrill et al. 1990). The virus has also been identified by RT-PCR in *Hyalomma* ticks of livestock origin (Sang et al. 2011). The first documented clinical case was confirmed in 2000 (Dunster et al. 2002). Repeated outbreaks are known to occur in neighbouring countries, such as Uganda (Kizito et al. 2018, Balinandi et al. 2018). The World Health Organisation (2017) considers Kenya to be an at-risk country for CCHF with serological and virological evidence, vector presence and under 5 cases reported yearly. However, it is likely that the reported case numbers do not represent the true disease burden (Temur et al. 2021).

4.4.2 Transmission in the enzootic cycle

The CCHF virus has been detected from various ixodid and some argasid tick species (Gargili et al. 2017). It should be noted that virus detection is not in itself proof of a vector species. Virus detection without actual vector competence is possible, for instance, after a recent blood meal from an infected vertebrate (Whitehouse 2004). To date, the virus in known to replicate only in ixodid ticks, *Hyalomma* spp. in particular, which act as both vector and reservoir for

CCHFV. The pathogen is maintained transovarially, transstadially and venereally persisting in the ticks throughout their lifespan (Papa et al. 2017). Viral replication takes place in the tick's midgut, later the infection spreads to other tissues. The salivary glands, which are the main route for host infection, will accumulate a high viral titre (Gargili et al. 2017). Tick saliva contains many immunomodulative agents which are designed to overcome host defence mechanisms and facilitate transmission of pathogens. In addition to immunomodulation, the salivary glands produce cement to ensure attachment to the host for the feeding period (Papa et al. 2017, Wikel 2014).

Transmission of CCHFV via vector route is dependent on the tick's life cycle (Figure 3). After hatching, transition from larvae to nymph requires a small vertebrate host to feed on (Gargili et al. 2017). CCHFV is passed on transstadially from infected egg to larvae and nymph (Papa et al. 2017). 2-host ticks moult into nymphs while still attached to the host, 3-host ticks will drop off to moult at this stage. Besides infecting their first hosts, the moulting ticks may also co-infect other larvae and nymphs (Bente et al. 2013). After dropping off and moulting into

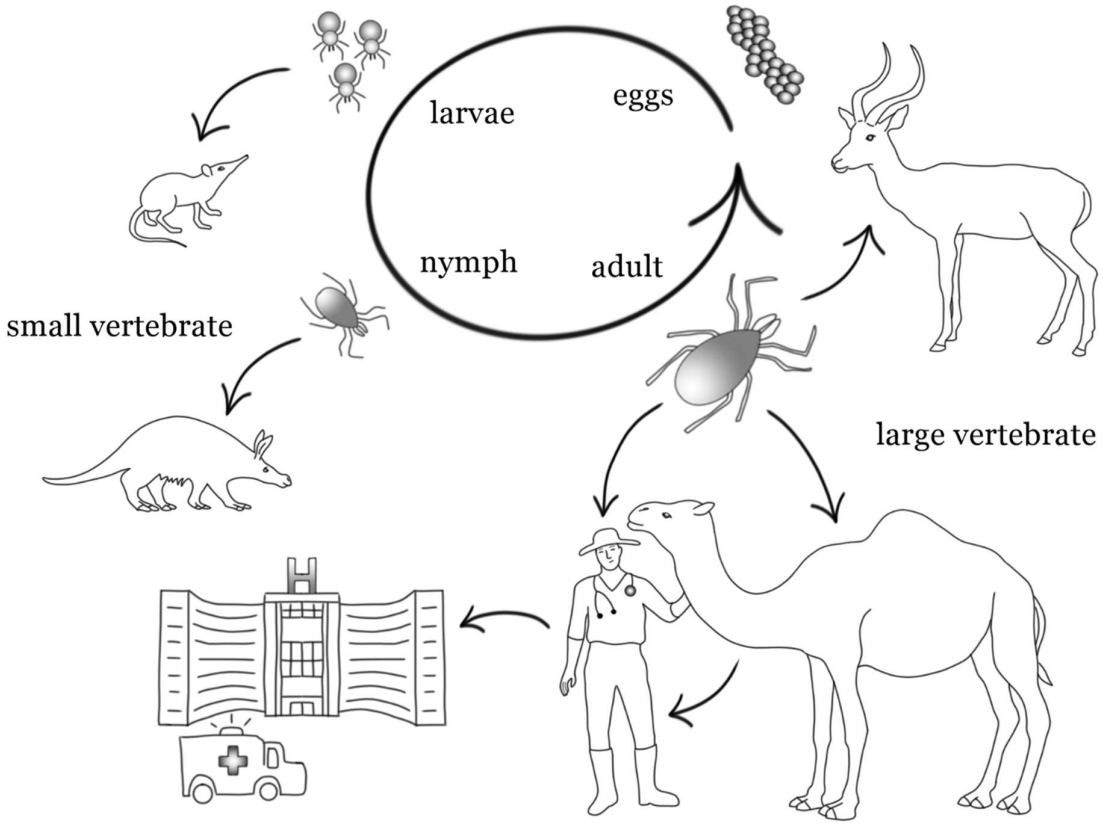

Figure 3. Exemplary life cycle of the *Hyalomma* spp. tick and the transmission routes of the CCHF virus.

adults, the ticks require a large animal as their second or third host for feeding and mating, depending on their life cycle. Again, besides infecting their large animal host, co-infections and venereal transmission occur between ticks (Gargili et al. 2017). The ticks may also infest humans, which are considered accidental, dead-end hosts (Bente et al. 2013). At the end of the cycle, the females proceed to drop off their host and lay their eggs, transmitting the virus transovarially to the next generation (Gargili et al. 2017).

4.4.3 Transmission in humans

In addition to tick bites, it is possible for humans to acquire the CCHFV infection through contact with the blood or tissues of viraemic animals, ruminants in particular (Gargili et al. 2017). Removing and crushing engorged ticks from farm animals, sheering tick-infested sheep and slaughtering animals are among high-risk activities (Spengler et al. 2016). Handling and consuming animal products such as uncooked meat and unpasteurised milk may also be a source of infection (Nasirian 2019). In Africa, the practice of backyard slaughter in variable circumstances and consumption of wild "bush meat" are thought to be risk factors for the transmission of haemorrhagic fever viruses (Kingsley 2016). Birds are generally considered to be refractory to the virus (Spengler et al. 2016). However, several human epidemics in ostrich farms and abattoirs in South Africa have provided contradictory information on the viremia of ostriches and the risk they pose to humans (Swanepoel et al. 1987, Swanepoel et al. 1998).

CCHF can spread between humans via blood or bodily secretions, especially in hospital settings. The risk for nosocomial infections is posed not only to patients, but also healthcare workers (Gozel et al. 2013). Possible exposure routes include blood transfusions, surgical procedures and needlestick injuries (Gozel et al. 2013, Nasirian 2019). Cases have been reported of infections occurring when taking care of family members who have fallen ill (Aradaib et al. 2010). No evidence of sexual transmittance between humans has yet been found, although possible in certain other VHFs (Blumberg et al. 2014). Infectiousness of aerosols is possible but under debate, in which case handling of patients would necessitate the use of respirators and negative-pressure isolation rooms (Nasirian 2019). However, the use of extensive personal protective equipment, barrier nursing routines and patient isolation are currently thought to be sufficient in preventing infections (Gozel et al. 2013, Nasirian 2019, Ftika & Maltezou 2012). These quarantine measures are subject to the education and equipment level of healthcare facilities

and their staff, and may be seriously impaired in resource-poor countries (Ftika & Maltezou 2012, Aradaib et al. 2010).

4.4.4 Ecology

Changes in the use of arable land and animal husbandry paired with other transformations in ecosystems such as deforestation have an impact on vector abundance and CCHF infections (Estrada-Peña et al. 2012). Due to the seasonal changes in tick populations most human CCHF infections are seen during the spring and summer. Based on tick survival overwinter, mild winters result in more infections during the summer and less infections are seen after very cold winters accordingly (Nasirian 2019, Estrada-Peña & Venzal 2007, Esser et al. 2019). Higher air humidity is a driving factor in tick survival, but the correlation with precipitation is unclear and most probably dependent on climate zones (Esser et al. 2019). Climate change will also affect the distribution of suitable vector species (Estrada-Peña et al. 2012). Increasing warmth generally favours tick survival but simultaneously changing precipitation patterns are poorly understood in relation to tick ecology. Rising temperatures paired with lower humidity might in fact lead to tick desiccation (Esser et al. 2019, Estrada-Peña et al. 2012). In Europe, the main vector genus *Hyalomma* spp. will likely spread to northern and western Europe (Estrada-Peña et al. 2013, Estrada-Peña & Venzal 2007).

4.5 Clinical signs

While becoming transiently viraemic, there is no evidence to date of the CCHF virus causing clinical manifestations in domestic or wild animals (Spengler et al. 2016). Humans, however, usually develop moderate to life-threatening symptoms and become viraemic after the first week of infection (Bente et al. 2013). The progression of clinical CCHF can be classified into four phases: incubation, pre-haemorrhagic, haemorrhagic and convalescence (Ergönul 2006). The incubation period of CCHF from infection to the onset of symptoms lasts 1-7 days and is slightly dependent on the mode of virus acquisition (Ergönul 2006). The pre-haemorrhagic phase begins with general symptoms such as fever, headache, nausea, vomiting and diarrhoea (Ergönul & Holbrook 2011). These can easily be confused with similar febrile diseases, especially in endemic areas, including other viral haemorrhagic fevers, rickettsial infections and malaria (Burt 2011). The pre-haemorrhagic phase, lasting for up to a week is followed by the short and rapidly developing haemorrhagic phase, where the initial symptoms worsen and

haemorrhaging may be detected in various locations (Ergönul 2006). CCHF is distinguished by the most severe haemorrhagic symptoms of all the VHFs (Peters & Zaki 2011). Bleeding under the skin, conjunctiva and the mucous membranes usually manifests as a petechial rash but may expand to large ecchymoses. Other common bleeding sites are the urinary, gastrointestinal and respiratory tract (Ergönul 2006). Uterine or vaginal bleeding is a known complication in pregnant women with CCHF, often resulting in newborn mortality (Pshenichnaya et al. 2017). Hepato- and splenomegaly are common, neuropsychiatric and cardiovascular changes have also been reported (Ergönul 2006, Bente et al. 2013, Whitehouse 2004).

Contrary to the name of the disease, death does not typically follow from profuse bleeding, but rather from a cascade of severe conditions, including disseminated intravascular coagulation, vascular damage, shock syndrome and multiple organ failure (Paessler & Walker 2013, Whitehouse 2004). Fatality rates range from 15 to 30 %, although significantly variable numbers have been reported (Peters & Zaki 2011, Whitehouse 2004). Initial viral load and the sufficiency of the antibody response may be key to the severity and lethality of the disease (Whitehouse 2004). If the patient survives the haemorrhagic phase, the symptoms start to ease within two or three weeks. During the convalescent period, patients usually make a full recovery, although experiences of prolonged malaise occur (Ergönul 2006). No latency or relapse of infection has been documented (Bente et al. 2013).

4.6 Laboratory diagnostics

There is no gold standard for CCHF laboratory diagnostics, but the molecular diagnostic approach for detection and amplification of viral RNA, mainly reverse transcription polymerase chain reaction (RT-PCR), is considered the first choice for acute-phase serum sample testing due to its speed, safety and sensitivity (Bente et al. 2013). Viral nucleic acids can be detected as early as 24 hours after infection, whereas detectable antibody production takes at least 4-5 days. Furthermore, immunological assays are often futile in those fatally ill patients, who do not develop an antibody response (Burt et al. 1994, Burt 2011). Rapid identification of the disease and quantifying information about viral load is essential in commencing treatment, controlling further infections and forming a prognosis (Ergönul 2006, Burt 2011). RT-PCR overcomes the lag time of antibody production and its individual variation and gives critical information about the severity of the disease (Burt 2011). Due to the genetic diversity of CCHFV,

the RT-PCR technique requires several primers and necessitates constant updating as the virus evolves through time (Tezer & Polat 2015). A shortcoming of the RT-PCR is that it is commonly performed in reference laboratories, stretching the acquisition of test results to days in certain endemic countries and areas (Al-Abri et al. 2017). New, rapid point-of-care diagnostic tools are therefore urgently needed for medical facilities with limited resources and for crisis situations where CCHF thrives (Burt 2011).

The immunological assays are based on the detectable specific antibody response within a week after the onset of the clinical disease (Tezer & Polat 2015). Currently the anti-CCHFV IgM or IgG based enzyme-linked immunosorbent assays (ELISA) is mostly used, but less sensitive immunofluorescence assays (IFA) and neutralisation tests have also been utilised in the past (Ergönul 2006, Burt et al. 1994). The specific IgM lowers to an undetectable level in months, whereas IgG may be present for several years (Burt 2011). IgM is therefore used for diagnosis of recent infections and IgG for surveillance purposes and retrospective diagnosis of survivors (Tezer & Polat 2015). It should be noted that PCR positivity decreases when the antibody response increases (Burt 2011). Ideally, RT-PCR and IgM ELISA tests should both be performed in acute patients when available (Bente et al. 2013). Viremia and antibody kinetics as well as timing for different diagnostic tools are summarized in Figure 4.

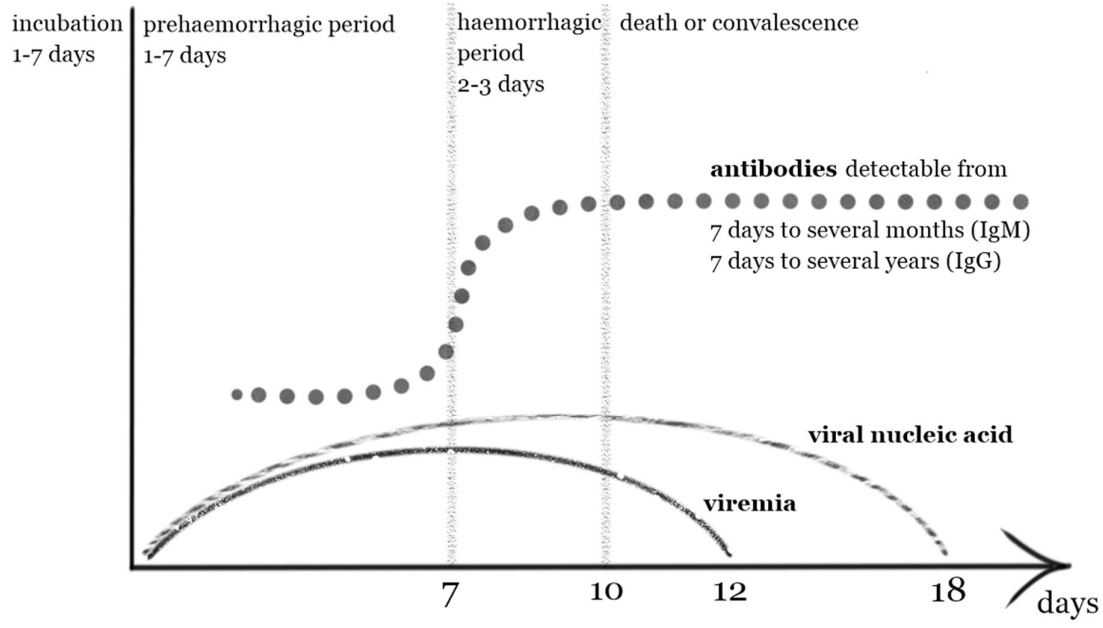

Figure 4. Simplified kinetics of viremia and antibody response (IgG/IgM) in relation to progression of the disease (Burt 2011, Ergönul 2006).

Due to the hazardous nature of the CCHF virus, immunological assays and molecular diagnostics are the basis for day-to-day diagnostics. CCHF viral isolation and culture attempts should be done in maximum biocontainment laboratories and are therefore used mainly for research purposes rather than patient diagnostics, although some endemic countries process samples in BSL-2 facilities as opposed to non-endemic countries with a BSL-4 level requirement (Weidmann et al. 2016). Blood samples, or alternatively, liver tissue suspensions from acute-phase patients or ticks may be cultured in newborn mice or cell cultures, where the latter is less sensitive (Burt 2011). Further identification of CCHFV is done by monoclonal antibodies and immunofluorescence assays (Zivcec et al. 2017). Observing the cytopathic effect (CPE) of the CCHFV is not reliable, since the virus is mainly noncytopathic by nature (Canakoglu et al. 2013).

4.7 Therapy and prophylaxis

Treatment of CCHF is mostly supportive, success depending heavily on early detection as no specific drug treatment exists (Ergönul & Holbrook 2011). Some cases are asymptomatic or mild and non-specific, where treatment is redundant. The supportive treatment measures in severe, life-threatening cases aim to reduce the risk of lethal complications such as disseminated intravascular coagulation, hypovolemic shock and multiple organ failure (Leblebicioglu et al. 2012). Intravenous fluid administration is necessary in patients with hypovolemia and hypotension, originating from increase in vascular permeability and vascular damage. Haemorrhaging patients, depending on severity, may receive blood transfusions in addition to IV-fluids (Leblebicioglu et al. 2012). Renal insufficiency and/or severe metabolic acidosis and respiratory failure may require haemodialysis and mechanical ventilation, respectively. Antibiotics can be given for severe secondary infections such as sepsis and pneumonia, paracetamol is used for analgesia and pyrexia (Leblebicioglu et al. 2012). It is standard practice to use the antiviral drug ribavirin as supportive treatment to reduce mortality and clinical signs, but evidence is mainly empirical and the conducted studies non-randomized and heavily confounded (Johnson et al. 2018, Soares-Weiser et al. 2010). In addition to treating clinically ill patients, ribavirin is usually offered in nosocomial exposure situations (Gozel et al. 2013). Experimental antibody therapies have also been carried out, with anti-CCHFV immunoglobulin from the plasma of survivors transferred to acute patients (Bente et al. 2013).

Bulgaria is currently the only country with a vaccine in use for CCHF, but it is not available anywhere else and has not passed clinical trials (Papa et al. 2011). The genetic diversity of CCHF and the stringent biosafety requirements make vaccine development challenging. Under experimental conditions, the virus has also failed to replicate in any other mammalian host other than newborn mice (Bente et al. 2013). In the absence of a treatment protocol or a vaccine, prevention of tick infestation in domestic animals, tick-bites in humans and accidental or nosocomial infections in at-risk professions form the basis of CCHF control (Whitehouse 2004). For humans, regular body checks for ticks and the use of repellents are an effective way of disease prevention (Ergönul 2006). The control of ticks in domestic animals has traditionally been done by acaricidal agents, although recently anti-tick vaccines have also been researched as a tool to limit the spread of CCHF and other tick-borne disease (Manjunathachar et al. 2019).

B - EXPERIMENTAL WORK

5 Aim of the study

Figure 5. A map of Kenya and the location of the study area, Taita Hills in Taita-Taveta County.

This licentiate thesis was a part of the larger "Preparedness for emerging zoonotic infections in Kenya" -project led by Professor Olli Vapalahti in the University of Helsinki, in collaboration with the University of Nairobi. The intent of the project was to investigate zoonotic disease prevalence, ecology and epidemiology in Kenya through human patients, wildlife and domestic animals. Additional objectives were increasing laboratory capacity for swifter diagnostics, implementing screening programmes and enhancing preparedness for zoonotic disease. The research sites included Busia County by Lake Victoria and the Ugandan border in western Kenya, the Kibera informal settlement in Nairobi, the country's capital in central Kenya, and the Taita Hills mountain range in Taita Taveta county in south-eastern Kenya (Figure 5).

The objective of this thesis was to investigate whether free roaming ticks could provide evidence of CCHFV circulation in the Taita Hills area. Taita-Taveta county is part of a biodiversity hotspot with a significant proportion of the land area dedicated to national parks, but also a

lively site for domestic animal rearing, forestry and other human interferences (Taita Taveta County Government 2018). In addition, the area is affected by wildlife declines, habitat loss and the consequent increase in ecosystem fragmentation (Ogutu et al. 2016, Tóth et al. 2014). These disturbances in natural cycles together with constant intermingling of domestic animals, wildlife and humans promote the emergence and circulation of zoonotic disease (Plowright et al. 2017, Wells et al. 2019). The area's rich flora and fauna also support a broad variety and high density of ticks suitable for CCHFV vector role (Kariuki et al. 2012, Cumming 2000). On these bases it was estimated that CCHFV might be found in the Taita Hills area, but currently data on viral presence is lacking.

For the study, a set of ticks collected from two conservation areas in the Taita Hills were processed to extract nucleic acids. The procedure yielded a DNA-phase and an RNA-phase, of which the first was stored for further research of diseases with a DNA-genome, and the latter was analysed by RT-qPCR in search of CCHFV. An additional conventional PCR panel was also conducted to find other viruses from the *Bunyavirales* order targeting nairoviruses and phleboviruses in general. These genera include the viral haemorrhagic fever viruses Nairobi sheep disease and Rift Valley fever, which are predominantly animal pathogens. However, this panel was less sensitive and was not aimed at species level.

6 Materials and methods

6.1 Tick collection

Figure 6. The collected ticks before grounding and RNA extraction. Photo by Tarja Sironen.

The ticks used for the RNA-extraction were collected in 2018 by the Vapalahti virology team operating from the University of Helsinki's Taita research station in Wundanyi, Taita-Taveta county. The first group of ticks consisted of free-roaming individuals collected from the territory, pictured in Figure 6. A collection of engorged ticks removed from domestic animals was carried out simultaneously, but these were not processed or examined in this licentiate thesis. The purpose of collecting ticks both from the environment and animals was to compare CCHF presence in ticks with and without blood meals inside them, since blood from viraemic animals interferes with determining true virus circulation and vector competence in ticks.

The ticks of the first group were arranged in a total of 57 tubes processed as units, with one tube containing 1-3 ticks of similar morphology and life-stage. Ticks forming 33 tubes were collected from the Lumo conservancy and 24 tubes from Sarova conservancy. At collection,

some characteristics, such as life stage, colour and sex of the ticks were observed and documented but they were not classified to species level. It was estimated that a large portion of the tick material was from the *Rhipicephalus* genus, with a few *Hyalomma* spp. The ticks were preserved in RNAlater stabilization solution (Thermo Fisher Scientific) and kept frozen in -20 ºC for optimal and longstanding protection of cellular RNA. Collection of ticks and the involvement of animals for the purposes of the study in Kenya was warranted by the Kenya Wildlife Service and the import licence to Finland was granted by the Finnish Food Authority.

6.2 RNA extraction

The RNA extraction of the free-roaming ticks was completed between September and November 2019. To release nucleic acids, the intact ticks had to be finely ground. This was achieved by moving the ticks to new 2 ml Eppendorf containers along with about 200 µl of sterile sand, a metal bead with a diameter of 5 millimetres and 900 µl of TriZol reagent. The mixture was then lysed in the TissueLyser II (Qiagen) device for 2 minutes with an RPM of 30 1/s. After lysing, the separation of the RNA, DNA and protein phase was performed according to the TRIzol™ manufacturer's (Invitrogen) instructions. After separation, the tube contained a lower, red-coloured phase (DNA and phenol-chloroform), a white-coloured interphase (proteins) and a colourless upper phase (RNA). The RNA-phase was pipetted to a new tube and the DNA-phase was left in the original tubes for future research of DNA-viruses and other bacterial and protozoal disease. The RNA was precipitated, washed and solubilized according to the manufacturer's instructions (Qiagen Viral RNA kit), with the exception of resuspending the RNA-pellet in the last stage in 100 µl of RNase-free water. This solution was then divided into two aliquots 50 µl each, thus leaving one extra aliquot for further research.

Finally, the RNA quantity from one set of the aliquots was measured with the NanoDrop 2000 (Thermo Fisher Scientific) spectrophotometer, in units of ng/nl. The RNA-extractions yielded a median result of 64,72 ng/nl, which was seen as adequate. A lower-than-expected amount of RNA might have resulted from the tube unit containing only one tick, or the tick being a nymph or a very small adult. In any case, the number of nucleic acids extracted from ticks is expected to be minimal compared to other materials, such as tissue and faecal matter. The 260/280 nm wavelength ratio, also known as the purity ratio for RNA/DNA, set into a median of 1,75. According to the manufacture, a ratio of 2,0 is seen as sufficient for RNA purity and 1,8 for DNA. Lower ratios may result from impurities from previous processes. The small RNA-yield might

have affected the low 260/280 ratio, since impurities disturb the outcome more drastically when handling minuscule amounts of RNA.

6.3 Real-time quantitative and conventional PCR

Real-time quantitative specific CCHFV PCR was performed according to the method developed by Jääskeläinen et al. (2014) with the exception of using 3 µl of RNA template instead of 7 µl. The primers and probes in this method targeted the S-segment of the CCHFV genome. The reactions were carried out by using the SuperScript™ III One-Step RT-qPCR System with Platinum™ Taq High Fidelity DNA Polymerase Kit (Invitrogen) in combination with the Mx3005p Multiplex Quantitative PCR System (Stratagene). For the control testing of the CCHFV PCR a set of three dilutions of a Turkish patient panel (Turkey 98) was used in addition to a negative control (water).

To widen the possible pathogen turnout from the samples a pan-nairo and pan-phlebovirus conventional PCR panel was run in addition to the CCHFV panel (Lambert & Lanciotti 2009). The test was performed with the MJ-Research PTC-200 Peltier Thermal Cycler following a thermal profile of 30 min at 50ºC (reverse transcription), 2 min at 94 ºC (enzyme activation), 15 s at 94 ºC, 55 cycles of 30 s at 55 ºC, 1 min at 68 ºC and lastly 5 min at 68 ºC (elongation). The same SuperScript™ III One-Step RT-qPCR System with Platinum™ Taq High Fidelity DNA Polymerase Kit (Invitrogen) was used as in the RT-qPCR. Finally, the amplified products were ran in an agarose gel by electrophoresis, picture of the PCR products are shown in Figure 7. The expected size of nairoviruses was 400 bp and 370 bp for phleboviruses. The control of this panel was done by the same protocol and samples as in the CCHFV panel.

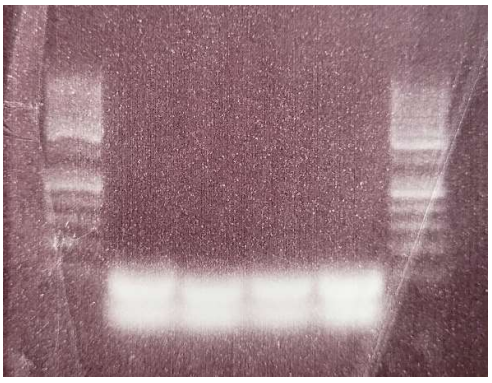

Figure 7. Pan-Nairo and pan-Phlebo agarose gel electrophoresis picture of the PCR products.

7 Results and discussion

7.1 Results

All tested samples came up as negative for CCHFV, as did the conventional panel for nairoviruses and phleboviruses. The controls worked, which indicated that the method itself was valid. The negative PCR results could have stemmed from very small RNA yields in the extraction process or the limited number of primers for the PCR, since CCHFV is genetically versatile. However, it may be concluded that no CCHFV was detected in the tick material. The processed free roaming ticks were less likely to contain large blood meals, which makes the results more relevant in terms of vector competence compared to ticks gathered from animals.

7.2 Earlier publications

Very few papers have been published indicating CCHFV prevalence in ticks in Kenya, especially lacking are studies made with questing or free roaming ticks. PCR screening of *Hyalomma*, *Amblyomma* and *Rhipicephalus* spp. ticks in north-eastern Kenya was performed by Sang et al. (2011), researching the presence of CCHFV in ticks gathered from livestock. Positive results were found among *Hyalomma* spp. collected from cattle and camels. A similar study was carried out by Chiuya et al. (2021) in Western Kenya, where various tick-borne pathogens were screened from ticks as well as fleas and lice collected from livestock markets and abattoirs. Interestingly, here *Rhipicephalus* spp. ticks yielded positives. Both studies identified the ticks to genus or species level. Some studies have screened questing ticks for *Bunyaviridae* and other arboviral orders in general, such as Mwamuye et al. (2017) in Shimba Hills National Reserve.

Other CCHF research in Kenya has been focused on serology, both human and animal. Anti-CCHF IgG seroprevalences (presenting past exposure to CCHF) of 23% and 14% were found in patients presenting with acute febrile illnesses in two healthcare centres in north-eastern Kenya (Lwande et al. 2012). Increased risk was associated with agricultural work and contact with donkeys. The study also found a single IgM positive patient, indicating current exposure to CCHF (e.g. active disease). Tigoi et al. (2015) discovered an overall IgG seropositivity of 25,6% in a study focusing on Midwestern and Eastern Kenya. Here, the female sex and contact with goats were identified as risk factors. In a wider study of 9 geographically diverse health

care facilities an IgG seroprevalence of 1,9% was found, in addition to an 0,4% IgM seroprevalence (Nyataya et al. 2020). The wildlife-livestock interface was examined by Obanda et al. (2021), screening buffalo and cattle sera from different habitats. An overall seroprevalence of 75,3% in buffaloes and 28,1% in cattle was observed. The seroprevalence for buffaloes was higher in closed wildlife systems and lower in systems integrating wildlife and cattle. The study suggested that cattle might reduce tick abundance and thus lower anti-CCHF IgG seroprevalence in buffaloes via their dispersion of acaricides into their surroundings.

7.3 Further research

The DNA-phase accumulated from the RNA extraction process could be used to screen DNA-viruses, protozoa and bacteria. Further testing of the remaining RNA-eluate might also be undertaken. Specific Nairobi sheep disease PCR could yield positive results since the preferred tick host for the disease is *Riphicephalus* spp. rather than *Hyalomma* spp. (Madder et al. 2013, Walker et al. 2003). CCHFV PCR results from the ticks collected from animals could be analysed in conjunction with corresponding blood sample serology. This would provide a unique comparison of CCHF prevalence in free roaming and blood-fed ticks. Prominent anti-CCHF IgG seroprevalences have been found in previous studies of febrile patients, therefore serological studies in the Taita-Taveta county health centres could provide important information on CCHF exposure and thus help navigate further tick research in the area.

Vector-mediated bacteria such as *Ehrlicia* spp., *Ricketssia* spp., *Anaplasma* spp. and *Coxiella burnetii* as well as protozoans (*Theileria* spp.), serving as both animal and human pathogens, have been more amply tested from both tick and animal samples in Kenya (Oundo et al. 2020, Mwamuye et al. 2017). These materials could be used utilised for CCHFV research as well, if both the DNA and RNA phases were extracted and stored. Parallel research of other pathogens causing acute febrile illness is also important, since information on the local disease burden aids preparation and diagnosis in health care facilities (Tigoi et al. 2015, Bower et al. 2019).

7.4 Discussion

The main objective of this research section was to screen free-roaming ticks collected from the Taita Hills area, south-eastern Kenya for the presence of CCHFV by means of PCR-technology.

This was done as part of a larger objective to examine zoonotic disease occurrence and preparedness in the area and in Kenya on a whole, as well as observe the interdependencies of disease ecology and epidemiology. The results indicate that in this material, randomly picked free-roaming ticks do not contain detectable amounts of CCHFV in the Lumo and Tsavo conservancies. It should be noted that the material was rather limited with only 63 units after pooling the ticks, so it may not give a comprehensive view of CCHFV prevalence. Absence of viral RNA in this material does not automatically imply the lack of disease circulation in the area.

Evidence of CCHF circulation or absence needs further support via human and animal virological and serological studies as well as screening ticks found both in the terrain and animals and identifying them to species level (Temur et al. 2021). In addition, quantitative data on abiotic and biotic variables such as precipitation and tick reproduction rates are needed (Esser et al. 2019). Although the worldwide circulation of CCHF seems to follow the *Hyalomma* tick distribution, the enzootic cycle is in its entirety more complicated than certain other tick-borne diseases, such as *B. burgdorferi* sensu lato in the *Ixodes* genus (Randolph 2008).

7.4.1 Climate and biodiversity

The varying geography of Kenya with mountains and larger bush planes give rise to many climatic regions. The limited existing research data gives some indication that Kenya's hot and arid climate zones might be most susceptible to robust CCHF circulation. Both positive ticks and positive human sera have been found in Garissa County in the arid desert and steppe areas of North-Eastern Kenya (Sang et al. 2011, Lwande et al. 2012, Tigoi et al. 2015). On the other hand, similar findings have also been observed in the tropical rainforest and monsoon areas of western Kenya near Lake Victoria, as well as temperate zones in central Kenya (Tigoi et al. 2015, Chiuya et al. 2021, Nyataya et al. 2020). The areas surrounding Taita Hills are an interesting mixture of three different climate zones: tropical savannah, hot and arid steppe and temperate zones with dry winters and warm summers. According to existing studies and the risk model of Messina et al. (2015), Taita-Taveta can be profiled as a high-risk location for the transmission of CCHF.

To complicate research, climate change and biodiversity loss are constantly changing the ecosystems and therefore the disease would require long-term surveillance. Kenya, among many other African countries has experienced massive wildlife decline and increase of livestock

numbers in the last three decades (Ogutu et al. 2016). Prior information suggests that the emergence of infectious diseases, such as CCHF, increases hand in hand with habitat fragmentation stemming from the expansion of human dwellings and agricultural premises in biodiverse environments (Estrada-Peña et al. 2010, Patz et al. 2004). Furthermore, climate change alters seasonal patterns and tick viability and favours tick-borne disease circulation in certain scenarios (Ogden & Lindsay 2016). Alongside healthcare preparedness and building laboratory capacity, zoonotic disease prevention in Kenya should include biodiversity protection and planning sustainable infrastructure suitable for particular ecological surroundings (Reaser et al. 2021).

7.4.2 Characteristics of the Kenyan population

Certain aspects of the Kenyan population are predisposing to tick-borne disease and highlight the importance of research of CCHF amongst other pathogens. Various nomadic tribes and pastoralist lifestyles make people susceptible to tick bites and contacts with animal blood. Herding animals in search of pasture aid importing cases locally and across country borders (Chiuya et al. 2021). The grazing lands of domestic animals frequently overlap with wildlife habitats, which further promotes disease circulation (Lankester & Davis 2016). Animals, being valuable sources of income, may be housed in the same premises as people. These populations should be informed about safe animal handling practices, such as to avoid removing and crushing ticks from animals with bare hands and hygiene precautions in slaughtering (Ng'ang'a et al. 2016).

The continuous conflicts in Eastern Africa have supplied Kenya with a sizeable flow of refugees from multiple countries, mostly Somalia, Ethiopia and Sudan (now separated into Sudan and South Sudan). The largest refugee camp Dadaab is situated in the hot and arid Garissa County, where CCHF circulation has previously been detected. Poor infrastructure, limited healthcare availability and malnutrition expose to infectious diseases, but refugees may also export CCHF cases themselves or via their livestock (Polonsky et al. 2013, Pigott et al. 2017).

In close-knit family units, sick individuals might also pose a risk to relatives tending to them (Aradaib 2010). The country’s pre-existing disease burden complicates diagnostics but may also cause comorbidity. Other endemic febrile infections can easily be confused with haemorrhagic fevers, and a simultaneous active tuberculosis and/or HIV-infection may worsen the outcome in all tick-borne diseases (Lwande et al. 2012, Thwaites 2014, Peters et al. 2014). The needs and special factors for different demographic groups should be considered in CCHF

prevention. In addition, the tacit knowledge of pastoralists familiar with the patterns of nature could be utilised in further research (Lankester & Davis 2016).

7.5 Conclusions

Crimean-Congo haemorrhagic fever has been recognized by the World Health Organization as a priority pathogen in need of immediate research and development. This has stemmed from the combination of a disease capable of triggering a public health emergency which is simultaneously improperly managed and understood worldwide. The dilemma is highlighted in resource-poor settings such as the African countryside. In many parts of Africa, very little of the elementary facts needed for preparedness and prevention are known. Sporadic infections and small epidemics affecting mostly rural areas and farmers may not spark interest such as worldwide influenza pandemics do, but CCHF causes severe suffering and mortality amongst underprivileged people and may have devastating effects on already vulnerable healthcare services.

Primary research on zoonotic pathogens is the foundation of understanding prevalence and behaviour of disease. Preparedness for zoonotic disease outbreaks requires data on all aspects of the enzootic cycle. This information is not only useful in a particular area but can also help understand disease ecology and epidemiology elsewhere. Published primary research on CCHFV in ticks has not been conducted previously in the Taita-Taveta area or in South-eastern Kenya in general. Even though the PCR-results were negative, this study was merely the first step to determine, whether CCHF is a feasible zoonotic threat in the unique ecosystem of Taita Hills. Furthermore, the results supplement previous research on the topic elsewhere in Kenya and help understand the bigger picture of CCHF prevalence in Kenya.

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