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Investigation of Crimean-Congo Hemorrhagic Fever Virus (CCHFV) in *Hyalomma* spp. ticks and evaluation of knowledge and practices related to ticks and CCHF among the Karamojong pastoralists in Moroto district, Uganda

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SUMMARY

Crimean-Congo Hemorrhagic Fever (CCHF) is a fatal disease in humans and one of the most wide-spread viral tick-borne diseases (TBD) with cases found in Africa, Asia and Europe. Ticks are the main vectors of the causative agent CCHFV, Crimean-Congo Hemorrhagic Fever Virus. CCHF presents with symptoms like fever, weakness, headache, and hemorrhagic signs (e.g. hematemesis and epistaxis). There are different tick species able to transmit the virus, where species within the genus *Hyalomma* spp. are the most common. In Uganda, ten human cases of CCHF have been confirmed since 2013, and the global awareness about the disease in increasing. In a non-published study by Balinandi *et al.* from 2017, antibodies towards CCHFV was found in serum samples from cattle in the Moroto district in North-Eastern Uganda, in 2017, where previously no human cases of CCHF have been documented. The aim for this study, was therefore to investigate the prevalence of CCHFV in *Hyalomma* spp. in this region. A secondary aim was to get a better understanding of what the local cattle owners know about ticks, TBDs and prevention in both animals and humans. This was done by interviews using a structured questionnaire.

In total, 504 *Hyalomma* spp. (n=485 *Hyalomma truncatum*, n=19 *Hyalomma rufipes*), were collected from cattle in two different sub-counties (Nadunget and Rupa) in Moroto district. In total 474 *Hyalomma* spp. were divided in pools of five or less, total nucleic acid was extracted and tested by qRT-PCR in search for viral RNA of the CCHFV. All tick samples analyzed were negative of CCHFV. This result does not necessarily mean that the virus does not exist in the region, but rather could suggest that other tick species can be responsible for transmitting the virus. Another explanation could be that the prevalence of CCHFV is very low and not enough *Hyalomma* spp. were tested. Description of two possible human cases of CCHF were reported during the interviews which strengthens the suspicion of CCHFV being present and causing disease in humans also in this region. During these interviews it got clear that the cattle herd owners want more help from the government to handle TBDs. It was prominent too that there is an apparent lack of knowledge of the potential severity of TBDs in humans and how to prevent them. Education programs and other prevention measures should be implemented for future improvements for the health care of both animals and humans.

CONTENT

ABBREVIATIONS

A	*Amblyomma*
ALT	Alanine transaminase
AST	Aspartate transaminase
B	*Boophilus*
BCS	Body condition score
CBPP	Contagious bovine pleuropneumonia
CCHF	Crimean-Congo hemorrhagic fever
CCHFV	Crimean-Congo hemorrhagic fever virus
DIC	Disseminated intravascular coagulation
DNA	Deoxynucleic acid
ECF	East coast fever
ELISA	Enzyme-linked immunosorbent assay
H	*Hyalomma*
IL	Interleukin
M	Moroto
MP	Moroto pooling
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PPR	Peste des petits ruminants
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
R	*Rhipicephalus*
RdRp	RNA-dependent RNA polymerase
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
Spp.	Species
TBD	Tick-borne disease
TBEV	Tick-borne encephalitis virus
TNF	Tumor necrosis factor
UVRI	Ugandan virus research institute

INTRODUCTION

Crimean-Congo Hemorrhagic Fever Virus (CCHFV) is causing the fatal human disease Crimean-Congo Hemorrhagic Fever (CCHF), including clinical signs such as fever, nausea, diarrhea, weakness and diverse hemorrhagic symptoms (Bente *et al*., 2013). The awareness of the disease has augmented the last 25 years, especially in Eurasia where almost no cases were reported in the year 1995 but thousands of cases in 2010. The virus has so far been found on three continents of the world, Africa, Asia and Europe (Kilpatrick & Randolph, 2012). The name Crimean-Congo is derived from the two different places it was originally described in the middle of twentieth century: the Crimean region in the former Soviet Union and Congo in Africa. The main vector and transmitter of CCHFV are ticks from the *Hyalomma* spp., even though the virus also has been found in other tick species (Hoogstraal, 1979).

Hyalomma spp. belongs to the family *Ixodidae* and are hard ticks. They are dark brown in color and are large in size compared to most other tick species (Anderson, 2002). Just as CCHFV, they are found in Africa, Asia and Europe, and lately the *Hyalomma* ticks tend to spread over larger areas in these regions by e.g. migratory birds (Estrada-Pena *et al*., 2012b).

Since the discovery of CCHF, over 20 human cases have been confirmed in Uganda, where at least 10 of these were confirmed in the last seven years (Hoogstraal, 1979; Balinandi *et al*., 2015; Kizito *et al*., 2017; Outbreak News Today, 2019). This study is looking at the prevalence of the CCHFV in *Hyalomma* ticks in the district of Moroto in North-Eastern Uganda. Prior to this study was performed, no cases of CCHF had been described in the Moroto district, even though the main vector *Hyalomma* spp. has been known to reside in the area (Walker *et al*., 2014) and antibodies of CCHFV have been found in serum samples from cattle in a non-published study by Balinandi *et al.* 2017. A secondary goal is to get more knowledge about what the cattle owners know about ticks and their diseases, e.g. how to distinguish different genera, tick management including tick prevention and how they want to treat TBDs in the future.

LITERATURE REVIEW

Ticks in general

Ticks belong to the order *Ixodida*, in the class of *Arachnida*. There are three families in the *Ixodida*: *Argasidae*, ticks with a soft body, *Ixodidae,* ticks with a rigid shield or scutum, and *Nuttalliellidae*, the most basal lineage of ticks *(*Mans *et al*., 2011*)*. It is not fully known, but it is estimated that ticks may have appeared approximately 225 million years ago in the late Paleozoic or early Mesozoic eras, parasitizing reptiles (Klompen *et al*., 1996). Ticks can be found in most regions around the world and have relatively large body sizes compared to other species in the same subclass *Acari* (e.g. mites). Ticks are highly specialized bloodsucking ectoparasitic arthropods, feeding on mammals, reptiles and birds. They can ingest enormous quantities of vertebrate blood, lymph or digested tissues relative to their body size (Anderson, 2002). Larva, nymph and adult (male and female) are the three active stages of a tick, and egg is a fourth, but inactive stage. Adults and nymphs have four pair of walking legs, but larvae have only three. The capitulum is the anterior part of the body, which includes mouthparts, sensory organs, cutting organs and the hypostome. The latter is an organ with numerous teeth used to anchor the tick to the host's skin (Parola & Raoult, 2001). Ticks have a complex cocktail of salivary components to prevent the host defense system to act on blood loss and develop inflammatory reactions. Anti-clotting, anti-platelet, anti-inflammatory, vasodilatory and immunomodulatory components are all present in the saliva of ticks (Francischetti *et al*., 2009). For ticks to be able to find their hosts and to communicate with other ticks, they have a variety of sensory organs. Hair-like structures (setae) are found all around, on the body, legs and mouthparts. Haller's organ is a cluster of gustatory and olfactory receptors found on the dorsal surface of the tarsus of the first leg (Parola & Raoult, 2001).

Two different strategies of finding hosts are used by ticks. One method is the ambush strategy where ticks use the vegetation to climb up and wait with their front legs out for a passing host. The hunter strategy is when ticks actively search for hosts nearby their habitat and run to attack them (Parola & Raoult, 2001).

Ticks molting and reproduction is regulated by blood ingestion and females can lay all from 200 to 23,000 eggs (Anderson, 2002). Generally, mating occurs on the host (Parola & Raoult, 2001). Three-host cycle is the most common cycle amongst ticks, but some species can also have one-host or two-host cycles. In a three-host cycle, a larva develops usually for several weeks inside an egg. After hatching, the larva seeks out a host to feed, and when fed it detaches from the host and hides somewhere, most likely in soil or vegetation where the larva molts to a nymph. The nymph goes through the same procedure, by finding a host to feed, detach and then molts, either to a female or male. Male ticks may feed and mate several times before they die (Walker *et al*., 2014). Female ticks in the *Ixodidae* family only feed once before it lays a batch of eggs, and when depleted dies, while female soft ticks will feed multiple times (Francischetti *et al*., 2009). The three-host is a slow lifecycle which may take six months up to several years to complete. A one-host cycle is much simpler, and in the same way as for a three-host cycle, eggs are laid on the soil and hatch after several weeks. The larva crawls on vegetation to quest for a host. When fed, the larva does not drop off from the host, but it molts on the host where the nymph continues the feeding, and then molts to an adult on the same host. In a two-host cycle, the larva and nymph feed on the same animal, but the adult quest for a new host. The life cycle for one-host ticks is faster than for the other lifecycles (Walker *et al*., 2014).

Ticks can transmit disease causing pathogens by horizontal transmission when a microorganism is spread between ticks and vertebrate hosts. They can also transmit pathogens from one stadium to the next (transstadial) or by transovarial (vertical) transmission where microorganisms pass from the female to the eggs and further on to the next generation, the larvae (Walker *et al*., 2014).

***Hyalomma* spp.**

The *Hyalomma* genus includes twenty-seven different species and belong to the family *Ixodidae* (Sands *et al*., 2017). Female ticks are usually bigger than males and the size of unfed ticks are large, 5 to 6 mm. Mouthparts are anterior, and eyes are always convex. Typical for *Hyalomma* species is that they have an orbit, a circular groove surrounding the eye. The scutum is dark brown and present on the dorsal side of the females while the males have ventral plates, usually three pairs on their ventral side (Figure 1). Both sexes have an anal groove posterior to the anus, and festoons even though they are unclear in fed females. The legs are slender, and they usually have pale rings (Walker *et al*., 2014). There are different ways to differentiate the *Hyalomma* spp. One is to look at the punctations on the dorsal side, another is by looking at the stigmas behind the fourth pair of legs. Other ways of identification are to compare the genital apertures on females or the ventral plates on males (Walker *et al*., 2014).

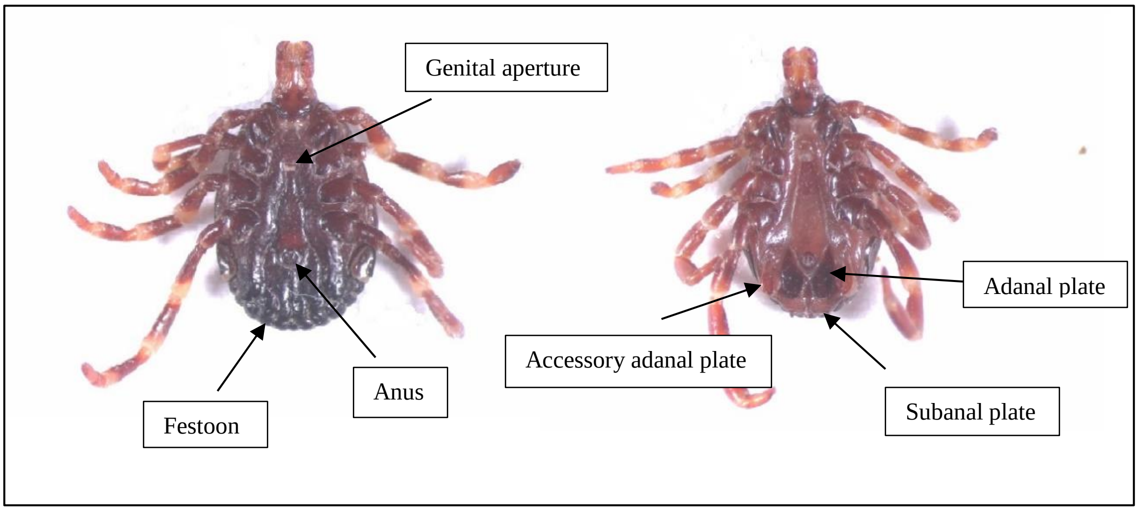

Figure 1. *Picture of ventral part of a female Hyalomma truncatum (left) and a male Hyalomma truncatum (right). Accessory adanal plate, adanal plate and subanal plate are the three ventral plates shown on the male. On the female showing festoons, anus and genital aperture.*

Hyalomma spp. are widely spread throughout Eurasia and Africa, and with them also CCHFV. *Hyalomma marginatum* is extending from Pakistan to Turkey and Kosovo and is the principal vector of CCHF in this area *(*Ozdarendeli *et al*., 2010). Eleven different species of *Hyalomma* are found in Africa, where four of them, *H. impeltatum*, *H. marginatum*, *H. rufipes* and *H. turanicum* are known to transmit the CCHFV *(*Walker *et al*., 2014*)*. *Hyalomma* spp. are known to have both "two-host" and "three-host" lifecycles depending on species (Bente *et al*., 2012). They are widely spread, and are found in different climate regions; forests, savannah and steppes and therefore shows a high tolerance of diverse climates (Hoogstraal, 1979). Recently, *H. rufipes* and *H. marginatum* have been found as far north as Sweden, and until 31st of October 2018, as many as 30 adult *Hyalomma* spp. ticks had been discovered around the country (SVA.se, 2018). Another important factor which may explain why *Hyalomma* spp. are so widespread, is because they are hunting ticks and can prey from a large variety of animals, including ground birds, hares, rodents and other small mammals for nymphs and larvae meanwhile adults actively attack livestock and other large animals (Hoogstraal, 1979).

According to Walker *et al*. (2014), there are three different species of *Hyalomma* in Uganda. Two of the species *Hyalomma truncatum* and *Hyalomma rufipes* (Figure 2 and 3) mainly feed on livestock (cattle, sheep, goats, horses) where the latter is more prone to transmit CCHFV. The third species, *Hyalomma dromedarii* mainly feed on camels, even though it can feed on livestock (Walker *et al*., 2014).

Figure 2. *Male Hyalomma rufipes (left) and male Hyalomma truncatum (right). The rufipes have punctations all around the dorsal side compared to the truncatum that mainly have punctations at the posterior part. Note: Pictures are not taken with same magnification.*

Figure 3. *Female Hyalomma rufipes (left) and female Hyalomma truncatum (right). The scutum has a slightly different shape and the rufipes have more punctations in the scutum compared to the truncatum. Note: Pictures are not taken with same magnification.*

In Europe, there are future concerns about climate change affecting the spreading of especially *H. marginatum*. Raised temperatures may increase the survival chance of the tick and the risk of transmitting diseases e.g. *Theileria* spp. to livestock or CCHFV to humans (Estrada-Peña, 2015).

Tick-borne diseases

There are around 900 different tick species around the world (Guglielmone *et al*., 2010), and it has been estimated that approximately 10% of these are vectors of pathogens (Jongejan & Uilenberg, 2004). Ticks are second to mosquitoes in the number of pathogens vectored to humans (Anderson, 2002). Protozoa, rickettsiae, spirochaetes, and viruses are among various of the transmitted disease-causing agents affecting humans, livestock, and companion animals (Jongejan & Uilenberg, 2004). The geographic range of many diseases are constantly expanding because of global factors such as, increased human migration, environmental change, insecticide resistance and expansion in global trading. Misdiagnosing of vector-borne diseases is common, particularly in developed countries where those diseases are not expected and the risk for vector-borne disease epidemics is greater now than it has been in the last fifty years (Ramalho-Ortigao & Gubler, 2020).

Lyme borreliosis (*Borrelia* spp.) is the most common TBD in North America, while ehrlichiosis (*Ehrlichia* spp.), rickettsiosis (*Rickettsia* spp.), Q-fever (*Coxiella burnetii*) and tularemia (*Fransciella tularensis*) are several other bacterial diseases transmitted to humans by ticks (Parola & Raoult, 2001). The two most known tick-borne viruses infecting humans are CCHFV and tick-borne encephalitis virus (TBEV), but there are to date at least 12 different genera of tick-borne viruses affecting humans and animals around the world (Shi *et al*., 2018*)*. Of the different TBDs, tick-borne viruses are causing the highest morbidity and mortality in humans (Jongejan & Uilenberg, 2004). The most common protozoal disease transmitted by ticks is babesiosis (*Babesia* spp.) mainly spread to cattle by *Rhipicephalus* spp. and *Ixodes* spp. Anaplasmosis (*Anaplasma* spp.) is another important disease affecting cattle transmitted by *Rhipicephalus* spp. (Merino *et al*., 2013). Of the world's livestock holdings, approximately 80 percent are affected of TBDs and in 2003 the estimated cost of TBDs was between $13.9 billion to $18.7 billion annually (Minjauw & McLeod, 2003*)*. In Africa, there are three main genera of ticks, *Rhipicephalus*, *Hyalomma* and *Amblyomma* responsible for most of these losses. Important diseases in livestock spread by these species are anaplasmosis, babesiosis, tropical theileriosis (*Theileria annulata*), heartwater (*Ehrlichia ruminantium*) and East Coast Fever, ECF (*Theileria parva*). *Theileria mutans* is another pathogen transmitted by ticks that facilitates the introduction of *Dermatophilus congolensis* responsible for significant losses in West Africa. Heartwater (cowdriosis) is a fatal disease that mainly affects sheep and goats, but also exotic cattle (Minjauw & McLeod, 2003). TBDs affecting pets are mainly a problem in the industrialized countries and tick-borne pathogens infecting horses have negative consequences for sporting events and international trade (Jongejan & Uilenberg, 2004). Dogs for example are susceptible to similar TBDs as humans including babesiosis, Lyme borreliosis, ehrlichiosis and anaplasmosis. Hepatozoonosis caused by the parasite *Hepatozoon* spp. is another tick-borne disease affecting dogs and other mammals (Elsheikha, 2016).

Since the 1990's the number of reported cases for tick-borne diseases Lyme disease, TBE and CCHFV have all had a major increase in humans. In North America, Lyme disease has almost doubled from 1995 to 2010. The same pattern goes for TBE in Europe during the period 1990 to 2005. CCHFV has had an even greater increase, with almost no cases reported in 1995, to over 1000 cases 2010 in Eurasia (Kilpatrick & Randolph, 2012).

Ticks and tick-borne diseases in Uganda

In Uganda there is a great variety of ticks, and thus also TBDs that can spread to humans and animals. In a study made by Byaruhanga *et al*., 2015, they were looking for ticks on cattle in the Karamoja region in North-Eastern Uganda and found eight different species, *Rhipicephalus appendiculatus*, *R. (Boophilus) decoloratus*, *R. pulchellus*, *R. evertsi evertsi*, *Amblyomma variegatum, A. lepidum, A. gemma* and *Hyalomma truncatum*, where *R. appendiculatus* was the most abundant species, 54.4% of the ticks. In the same study they found seroprevalence of *T. parva* (14.6%) causing ECF and *A. marginale* (86.6%), causing anaplasmosis. In addition, heartwater was diagnosed in two herds and babesiosis in one (Byaruhanga *et al*., 2015). In Entebbe, south of the capital Kampala, *R. simus, R. compositus and H. rufipes* were found in 1975-1977 amongst other already mentioned ticks (Kaiser *et al.*, 1982). The TBDs of most important economic value are ECF spread mainly by *R. appendiculatus*, anaplasmosis and babesiosis for the most part by the *R. (B) decoloratus* and heartwater transmitted by the *A.*

variegatum. ECF causes serious debility, morbidity, mortality and production losses and exerts the greatest limitation to improved production by cattle in Uganda (Okello-Onen *et al*., 1998). Other tick-borne pathogens found in Uganda from ticks collected on livestock are *Coxiella burnetii* causative agent of Q-fever and *Rickettsia* spp. causing rickettsiosis. In the same study *Rickettsia africae* was also discovered, the agent responsible for African tick-bite fever (Nakayima *et al*., 2014). CCHFV, the most geographically widespread tick-borne viral infection is another pathogen spread by ticks in Uganda and even though it does not have a major effect on animals, it is a mortal disease in humans. *Hyalomma* spp. is the main tick vector maintaining the disease in nature, but in Uganda viral RNA has been discovered in *R. (B.) decoloratus* (Balinandi *et al*., 2018). In the Karamoja region of Uganda, pastoralists consider TBDs to be of most importance regarding cattle production (Byaruhanga *et al*., 2015, Chenais & Fischer, 2018).

CCHFV

Crimean-Congo Hemorrhagic fever virus (CCHFV) is a member of the *Orthonairovirus* that belongs to the family *Nairoviridae* (Hoogstraal, 1979, Ergonul. 2006). CCHFV is a negative sense, tri-segmented single stranded RNA-virus. The segments are encapsidated by the nucleo-protein and RNA-dependent RNA polymerase (RdRp) (Zivcec *et al*., 2016). The shape of the CCHFV virion is spherical and approximately 80-100 nm in size (Bente *et al*., 2013).

As for all viruses, CCHFV is an obligate intercellular organism and relies on entering target cells for replication (Simon *et al*., 2009). The virion binds to cellular receptors with aid of glycoproteins Gc and Gn behaving as spikes in the lipid envelope (Bente *et al*., 2013) and enter host cells by clathrin-dependent endocytosis (Simon *et al*., 2009). Replication occurs inside the cytoplasm. Encapsidated genome segments interact with viral RdRp and synthesizes complementary positive-strand intermediates. These intermediates are used as templates to synthesize the negative-strand (Bente *et al.,* 2013). The virion is assembled at the Golgi membrane, and when the replication is completed, the new CCHFV buds off through the plasma membrane (Shayan *et al*., 2015).

CCHFV is characterized by tick-borne maintenance and transmission (Zivcec *et al.,* 2016). Infection of wild and domestic vertebrates with CCHFV causes a brief viremia and can serve as amplifying hosts (Spengler *et al*., 2016). Ticks maintain a life-long infection of CCHFV. The virion is transmitted to humans by tick-bites, by handling contaminated blood or tissues and may also spread from person to person (Mehravaran *et al*., 2013).

The distribution of the CCHFV includes Africa, Asia and Europe. In Africa the CCHFV has been isolated from at least nine different tick species, *Hyalomma anatolicum, H. impeltatum, H. impressum, H. rufipes H. nitidum, H. truncatum, Amblyomma variegatum, Rhipicephalus pulchellus* and *R. decoloratus* (Hoogstraal, 1979). Until 2010, the virus in Europe was limited to the Balkan region, but in the last ten years, RNA of the virus has also been detected in humans in Spain, and in migratory birds in Italy (Mancuso *et al.*, 2019). As mentioned previously, *H. marginatum* is the tick responsible for most cases of CCHF in Eurasia, even though the virus has been detected in a few other ticks in Turkey such as *R. bursa, R. turanicus* and *Ixodes ricinus* (Albayrak *et al*., 2010). Livestock as well as small animals have been shown to be exposed of CCHFV. Small rodents, ground squirrels, hares, cattle, hedgehogs, horses, ostriches, sheep and goats are all animals that have had experimentally documented viremia of CCHFV (Spengler *et al.,* 2016). The geographic distribution of CCHFV is constantly changing from year to year and is depending on weather conditions, the availability of vertebrate hosts, changes in vegetation and other factors affecting the success of molting and egg production of ticks (Estrada-Peña *et al.,* 2012).

CCHF the disease

The infection has been reported in wild and domestic vertebrate species but only causing an asymptomatic viremia lasting from 7 to 15 days (Spengler *et al*., 2016). The disease is fatal in humans and cases have been reported in Africa, Asia and Europe (Ergonul, 2012). CCHF causes a severe hemorrhagic fever in humans, characterized by fever, weakness, myalgia and hemorrhagic signs (Spengler *et al*., 2016) and should be suspected when a person shows these symptoms and has an appropriate exposure history. Furthermore, CCHF can cause physical findings suggestive of vascular leak and coagulation defects or nonspecific signs (nausea, vomiting, diarrhea, etc.). Together with laboratory evaluation showing thrombocytopenia, leukopenia, and elevated serum AST (aspartate transaminase) and ALT (alanine transaminase) levels, the suspicion of CCHF should be strengthened (Bente *et al*., 2013). Various forms of hemorrhage, including petechiae, large ecchymoses, melena and hematemesis tend to be more prominent in CCHF than in other viral hemorrhagic fevers. Disseminated intravascular coagulation (DIC), bleeding and shock can progress rapidly in severe cases. Proinflammatory cytokines including tumor necrosis factor (TNF)-alpha and interleukin (IL) 6 has been showed to be high, especially in fatal cases (Ergonul, 2008). The mortality rate of CCHF differs in studies but has been estimated to be from 9-50% in hospitalized patients (Shayan *et al.,* 2015).

Diagnosis of CCHF

Diagnosis of CCHF can be performed by different methods. Viral isolation by culture is the most definitive way to diagnose CCHF, but it is a slow process and takes 2-10 days to grow and may therefore be too slow in acute cases. It also necessitates a bio safety level 4 laboratory (Ergonul, 2012). Direct molecular detection by reverse transcriptase polymerase chain reaction (RT-PCR) is used to find viral RNA and due to its speed, sensitivity and safety should be considered the standard diagnostic method in acute clinical setting of disease (Bente *et al*., 2013). Indirect serological analysis by detection of specific IgM and IgG antibodies by enzyme-linked immunosorbent assay (ELISA) in human serum or blood is another way to detect infection of CCHFV. Analysis by ELISA has a sensitivity of more than 90% and is the most common technique for CCHFV antibody detection (Ergonul, 2012). New methods for diagnosis are constantly evolving, i.e. by MassTag PCR. A method has been designed with multiplex assays to rapidly differentiate different viral hemorrhagic fevers (Palacios *et al*., 2006). Rapid detection diagnosis tests have been designed for hemorrhagic fevers and hopes are to be able to get results within 30 minutes in the field without electrical power or expensive and sensitive equipment (Lucht *et al*., 2007). Rapid diagnosis test is based on detection of antibodies in the field. A test has recently been tried out for IgM antibodies of CCHFV by samples from Iran which showed a specificity of 92.9% and a sensitivity of 39.7% (Baniasadi *et al*., 2019).

Treatment of CCHF

Most infections of CCHFV are asymptomatic or result in a nonspecific febrile illness without the need of hospitalization (Bente *et al*., 2013). In the small percentage of patients developing clinical symptoms like hypotension and hemorrhage, early diagnosis is critical for patient support and for preventing spread of infection through well-documented human-to-human transmission (Spengler *et al*., 2016). The protocol for treating CCHF is currently based on general supportive measures, the use of the drug ribavirin and monitoring of the patients hematologic and coagulation status (Ergonul, 2008). Supportive therapy should include, histamine receptors blockers for peptic ulcer patients, monitoring of fluid and electrolyte balance, and if needed, administration of thrombocytes, fresh frozen plasma and/or erythrocyte preparations. Ribavirin is a synthetic purine nucleoside analogue and it inhibits the replication of a wide range of DNA and RNA viruses in vitro (Ergonul, 2008). Ribavirin is commonly used on its own against Lassa fever, and together with interferon on patients with Hepatitis C. For patients exposed to CCHF, ribavirin is also used for healthcare as a post-exposure prophylaxis (Johnson *et al*., 2018). It has not been determined how the mechanism of ribavirin acts in cases of CCHF and studies suggest giving ribavirin in early stages of disease may reduce mortality (Ergonul, 2008). The use of ribavirin is not uncontroversial. The drug has been demonstrated to have an inhibitory effect on the replication of CCHFV in vitro and in vivo, but the efficacy of ribavirin in treating CCHF has not been proven (Shayan *et al*., 2015). In a Turkish study from 2010, it was demonstrated that there was no positive effect on clinical or laboratory parameters in ribavirin-treated CCHF-patients. The study actually showed that leukocyte levels took longer to return to normal than patients not treated with ribavirin. Consequently, ribavirin had an observed negative effect on recovering from CCHF (Koksal *et al*., 2010). There have been two vaccines developed, but none have undergone official randomized clinical trials and their efficacy is questionable (Shayan *et al*., 2015).

CCHFV in Uganda

In Uganda, the most known tick species to transmit CCHFV is *H. rufipes*. *H. impeltatum* has also been reported to be able to spread CCHFV to humans, and it exists in Western Kenya, to the boarder of Eastern Uganda (Walker *et al*., 2014). In 2015, viral RNA of CCHFV has been found in a *R. (B.) decoloratus* (Balinandi *et al*., 2018).

The first case of CCHFV in Uganda was discovered in 1958, and between 1958-1977, 12 cases were described, with a fatality rate of two thirds (Hoogstraal, 1979). Three different outbreaks occurred in 2013, where six different cases were confirmed. Another case of CCHFV was reported and confirmed in November 2015, when a para-veterinarian got sick in the Nakaseke district in Uganda. The source of the disease could not be clearly identified, even though a brown tick was suspected (Balinandi *et al*., 2018). In August-September 2017, there were two new unrelated outbreaks of CCHFV in the central parts of Uganda, in the Kyankwanzi and Nakaseke districts (Kizito *et al*., 2017). The last known case was confirmed 31st of July 2019, in the Lyantonde district and was involving a 42-year old businessman who was buying and selling cattle (Outbreak News Today, 2019). At the time of the study, as far as literature goes, no known cases of CCHFV have so far been described in the Moroto district, in North-Eastern Uganda.

MATERIALS AND METHODS

Sampling areas and study population

Moroto district is located in the North-East of Uganda, in the Karamoja sub-region and consists of mainly vast plain and is limited by the Moroto Mountain to the east, belonging to a chain of volcanoes along the Ugandan international border to Kenya. Two major tribes inhabit Moroto, the Karamojong who live on the rangelands, and the Tepeth, living in the mountains of Moroto and Napak. Both tribes are nomadic pastoralists depending on cattle and subsistence crop production as their livelihood. Their culture is rich, and they dress, talk and live in traditional ways (Moroto district, 2018). The climate in Moroto is classified as tropical with an annual temperature of 21.9°C. Summer time is significantly wetter than winter time, with rainy season ranging from April to August (Climate-data, 2019). Northern Uganda is the poorest region of Uganda where the Karamoja sub-region has the highest poverty rate of all, at a rate of 74%. In Moroto district the poverty rate is estimated to be between 55,1%-75.0% (Uganda Bureau of Statistics, UNICEF & the World Bank, 2018). In 2005, the literacy rate of Karamoja sub-region was only 20% compared to the national average of 68% (Okech, 2006). Sampling areas were in two different sub-counties, Nadunget and Rupa in the Moroto district. The areas were selected based on the non-published study by Balinandi *et al.* 2017, finding antibodies of CCHFV in serum samples from cattle. A few herds were chosen as hot spots (Pupu and Nadunget parish), because *Hyalomma* spp. had previously been found in these areas in the same above mentioned study. The interviews were done with people from the Karamojong tribe and the different herds where ticks were collected were selected by two local veterinarians. One veterinarian for each sub-county. The research project has been ethically approved by School of Veterinary Medicine and Animal Resources Research Ethics Committee, Makerere University, Uganda (SVARREC/03/2017) and Uganda National Council for Science and Technology, Uganda (A580).

Questionnaires

To collect data on the perceptions of the cattle owners regarding TBDs, we interviewed a total of 50 cattle owners, 25 respondents from each sub-county. The interviews were done from 20-28th of September 2019. A structured questionnaire with 43 questions was used for the interviews with the cattle herd owners. The questions covered topics such as basic information about the person interviewed, knowledge and practices related to ticks, diseases and prevention of ticks and diseases in both animals and humans. The questionnaire was evaluated after the first day and it was changed to instead include 37 questions (Appendix A). The improved questionnaire contained 15 open questions and was used from the second day. In order to simplify the identification, the old name for *Boophilus* spp. was used instead of the new name *Rhipicephalus* spp. to avoid confusion from other *Rhipicephalus* spp. As part of the questionnaire, picture of ticks, both in black and white and in color as well as newly collected ticks were shown to the respondent. To be interviewed, each person had to sign a consent form to be able to join the study (Appendix B). The interviews were done with people of the Karamojong tribe, in the local language, Karamojong, together with a local veterinarian who acted as a translator. For some questions, when the owners did not understand, clarification of the questions had to be done. The answers from the participant was translated by the translator and another person was then recording all the answers by hand. The same person did all the recording throughout the study as well as the data analysis and interpretation. During the first two days, other villagers also helped to answer in a few interviews. This approach became too incoherent, and thus after two days only individual herd owners were interviewed separately

Tick collection

At the same as the interviews, two randomly selected animals from each herd were examined by a veterinarian, (the same person examined all chosen animals) and collection of as many ticks as possible with special interest of *Hyalomma* spp. Other tick species were also collected, for future studies. In total ticks from 102 cattle and 7 goats were collected. In one village four cattle were sampled from two different Kraals, but a second interview was not possible because the herd owner was standing next to the first interview and overheard the questions and answers.

During the field work, *Hyalomma* spp. were discovered in great numbers under goat tails, which is why a random number of ticks also were collected from goats.

The ticks were picked from the cattle with focus on the body parts that are close to the ground, i.e., tail, udder, lower body and legs, because this is the most likely place to find the hunting *Hyalomma* spp. They were picked from the cattle, the goats or the environment with forceps or handpicked with help of the locals. *Hyalomma* spp. are known to attack their host animal and not wait on grass straws like other species which is why ticks were looked after in the environment, especially in the areas where the cattle stay overnight (Kraals). The ticks from the environment were collected for the purpose of finding *Hyalomma* spp. that are not contaminated from sucking blood of the cattle. Ticks were collected from 20-28th of September 2019, and it rained for a couple of hours every day throughout the period of sample collection, ordinarily meant to be a dry season. The collection was done in the early mornings and late evenings in Nadunget sub-county, and only in the mornings in the Rupa sub-county. During daytime the animals were released for grazing and often too far away for proper sampling.

The ticks were placed and stored in 50 ml tubes, one tube per animal and *Hyalomma* spp. from the environment and goats were stored in separate tubes. Within three hours, the tubes were filled with 70% ethanol and stored in a dark dry place. The majority of the *Hyalomma* spp. sampled from the last two days of collection were stored alive in 50 ml tubes, opened twice a day in order to refresh the air, for maximum two days. They were then transferred and stored in a freezer of -80°C at the Uganda Virus Research Institute (UVRI). These samples were needed to find possible live CCHFV in the ticks. Unfortunately, some of the live ticks died during the journey, but were still placed in the freezer together with the others.

Animal health evaluation

A basic health evaluation was done of the 102 cattle included in the study. They were examined for; sex, breed, age, weight, body condition score (BCS), health status and tick burden estimate. Each cattle were assigned a special code, MXX, where M stands for Moroto. Weight was evaluated with a weight band, age by checking the teeth or asking the owner. BCS was evaluated using a 5-scale chart and health status by a basic veterinary examination. The ticks on one side of the animal were counted manually and when many, approximated. The health evaluation was done and noted by a Ugandan veterinarian experienced in working with cattle and ticks.

Laboratory analyses

All the ticks were taken to the UVRI in Entebbe, where each tube of ticks was separately opened and analyzed. Each tick was at first identified macroscopically by two qualified persons, and put in five different categories, male *Amblyomma* spp., female *Amblyomma* spp., *Rhipicephalus (Boophilus)* spp., *Hyalomma* spp. and *Rhipicephalus* spp. The identification was made on a petri dish on a chill plate. The ticks were then transferred to new 1.8 ml vials. Depending on sizes and species of the ticks between 1-99 ticks were stored per vial. Ethanol was once again added. Each sample contained strictly one of the above categories and was labeled after the cattle ID, species, genus and amount of ticks in vial. The live collected ticks, stored in the freezer, were macroscopically identified as *Hyalomma* spp. in the field and were identified by microscopy at the UVRI.

The microscope used was a Leica S6E, with magnifying capacity of 0.63-4 times (Figure 4). A Fiber-Lite M1-150 lamp was used for light to see the ticks clearly.

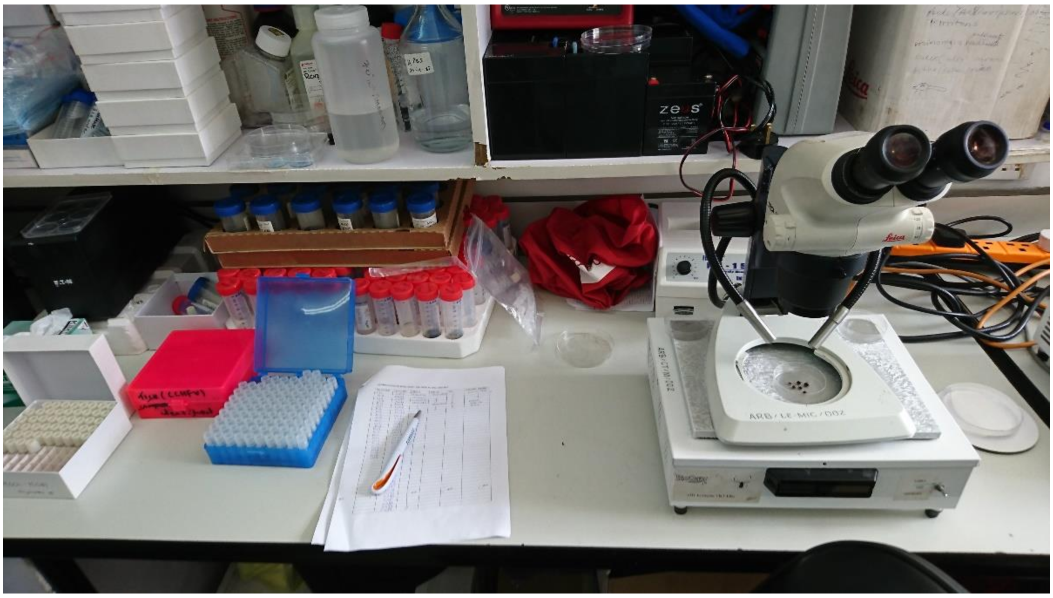

Figure 4. *A Leica S6E on a chill plate with a Fiber-Lite M1-150 lamp behind. Boxes with small 1.8 ml vials in front of the 50 ml bottles used in field. The sheet was used to note the amount of ticks for each sample.*

All the *Hyalomma* spp. ticks were examined one at a time. The ticks were first kept on a petri dish with ethanol on a chill plate, and then placed on another petri dish on the chill plate and identified by microscope observation of dorsal and ventral sides. When needed, the ticks were cleaned from dirt with the use of ethanol and a toothbrush on a separate petri dish. Their features were identified by comparing the different *Hyalomma* ticks, using the guide "Ticks of Domestic Animals in Africa: A Guide to Identification of Species" by Walker *et al*., 2014. Each tick was noted by genus, species, sex, and size. The females were subjectively defined, fully engorged, partly engorged or not engorged, while the males were subjectively defined, large, medium or small depending on size and the placement of subanal plates. Notes were made of ticks when special or different features were observed compared to what is normally seen. After identification of species, each tick was put separately in a 1.5 ml vial filled with ethanol and marked from M001 to M502.

The frozen ticks were kept on ice, before being analyzed on a chill plate. They were firstly cleaned with ethanol before identification, and afterwards rinsed with Phosphate Buffered Saline (PBS). Then they were put back in separate 1.5 ml vials and kept in the ice storage. When finished they were transferred back to the freezer of -80°C to keep the cool chain intact.

Pooling

Ticks were as far as possible pooled in pools of 5, but for a few cases they were pooled less than 5. *Hyalomma* ticks were pooled by using four different categories with a descending order of importance; 1) species, 2) sex, 3) geographical location and 4) engorgement/size. The ticks were strictly pooled with regard to the same species, sex and sub-county (Nadunget or Rupa) but in most cases mixed with *Hyalomma* spp. from different villages and herds. When it was possible the ticks were put together with other ticks of the same engorgement/size, otherwise they were mixed. The pooled ticks were placed in new 1.5 ml vials filled with ethanol.

Quantitative reverse transcription PCR

The ticks were kept in the freezer of -80°C at UVRI for a month, before analysis. The pooled samples were analyzed by local authorized laboratory personnel in a grade 4 security laboratory at UVRI, Entebbe, Uganda. The pooled ticks were placed in grinding 2 ml vials, containing 250 µL of a 1:1 ratio of isopropanol (MagMax Lysis Buffer) to MagMax Lysis Binding Solution (Life Technologies, Grand Island, NY). Using a GenoGrinder 2000 (OPS Diagnostics, Lebanon, NJ) the samples were homogenized for 2 minutes at 1,500 strokes per minute. Thereafter 550 µL of MagMax Lysis buffer was added, and the samples were transferred to cryovials and then immediately stored under liquid nitrogen vapors. A MagMax Pathogen RNA/DNA Kit (Life Technologies, Grand Island, NY) was used on a MagMax Express-96 Deep Well Magnetic Particle Processor (Life Technologies, Grand Island, NY) to extract nucleic acid. Quantitative-reverse transcriptase-polymerase chain reaction (qRT-PCR) were analyzed on all samples on a 7500 Real-Time PCR System (Life Technologies, Grand Island, NY) using the SuperScript III Platinum One-Step qRT-PCR Kit (Life Technologies, Grand Island, NY), with CCHFV-specific in house primes and probes. The primer and probe sequences used were:

Probe: 5'-/56-FAM/ACG CCC A/ZEN/CA GTG TTC TCT TGA GTG TTA GCA /31ABkFQ/-3'

Forward prime: 5'- CAA AGA AAC ACG TGC CGC TT -3'

Reverse prime: 5'- ATT CAC CTC GAT TTT GTT TTC CAT -3'

The extraction process was validated by performing a 16S ribosomal RNA (rRNA) on all the tick pools. Once again, the SuperScript III Platinum One-Step qRT-PCR Kit was used with four primers and probes, (Appendix C).

Both the qRT-PCR and the extraction process were including a positive control, a negative control and a non-template control (NTC).

RESULTS

Tick collection

In total 6524 ticks were collected and macroscopically identified as female *Amblyomma* spp., male *Amblyomma* spp., *Rhipicephalus* spp. or *Hyalomma* spp. (Table 1). Of these, 504 ticks belong to the *Hyalomma* spp. and all *Hyalomma* ticks were adults.

Table 1. *Summary of all the collected ticks*

***Amblyomma* spp. male**	***Amblyomma* spp. female**	***R. Boophilus***	***Hyalomma* spp.**	***Rhipicephalus* spp.**	**Total**
2738 (42.0%)	1748 (26.8%)	53 (0.8%)	504 (7.7%)	1481 (22.7%)	6524

During microscopical analysis of the *Hyalomma* spp., two different *Hyalomma* species were discovered, *H. truncatum* (n=485, 96.2%) and *H. rufipes* (n=19, 3.8%). In total 266 (52.7%) ticks were male and 238 females (47.3%) (Table 2).

Table 2. *Summary of collected Hyalomma spp. including species, sex and sizes (S, M and L are small, medium and large size)*

	S (%)	**M (%)**	**L (%)**	**Not engorged (%)**	**Partly engorged (%)**	**Fully engorged (%)**
H. rufipes male	1 (0.2%)	8 (1.6%)	8 (1.6%)			
H. rufipes female				0	2 (0.4%)	0
H. truncatum male	163 (32.3%)	86 (17.1%)				
H. truncatum female				84 (16.7%)	137 (27.2%)	15 (3.0%)
Total	**164 (32.5%)**	**94 (18.7%**	**8 (1.6%)**	**84 (16.7%)**	**139 (27.6%)**	**15 (3.0%)**

qRT-PCR-analysis

In total 100 pools of Hyalomma spp. were run for qRT-PCR analysis including 474 of the 504 collected ticks. All tested pools came out negative of CCHFV. Below is the plot of pool MP51-MP100 (Figure 5).

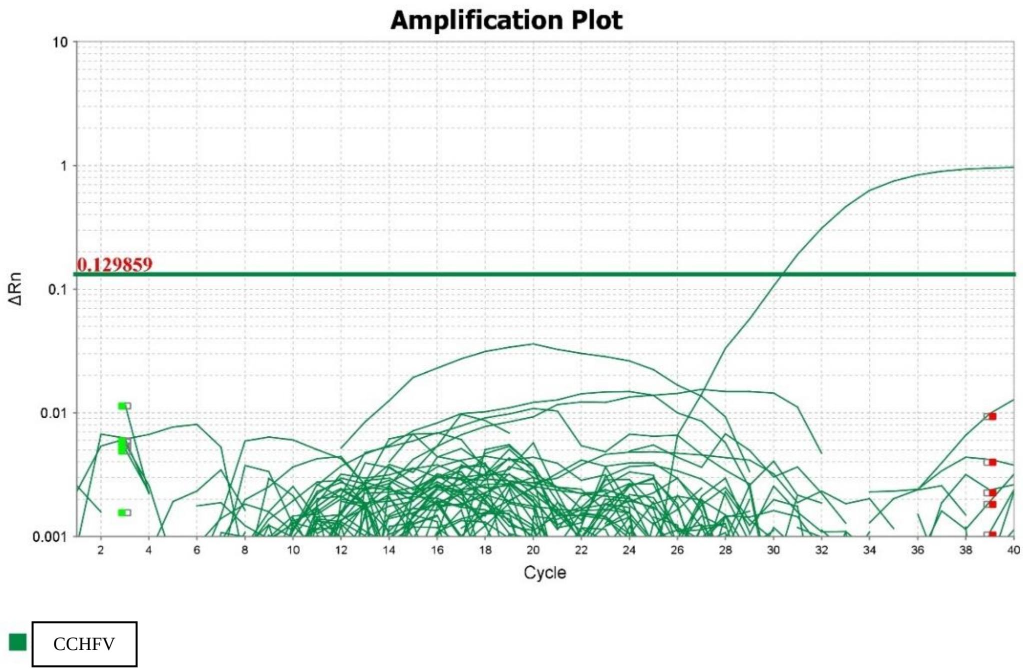

Figure 5. *The results of the qRT-PCR for CCHFV in the Hyalomma spp. The only curve crossing the reference line is the positive control.*

Below is the plot showing the results of the validation of the RNA extraction. Tick RNA was found in all samples by testing for 16S rRNA, which confirms that the method of extraction was successful (Figure 6).

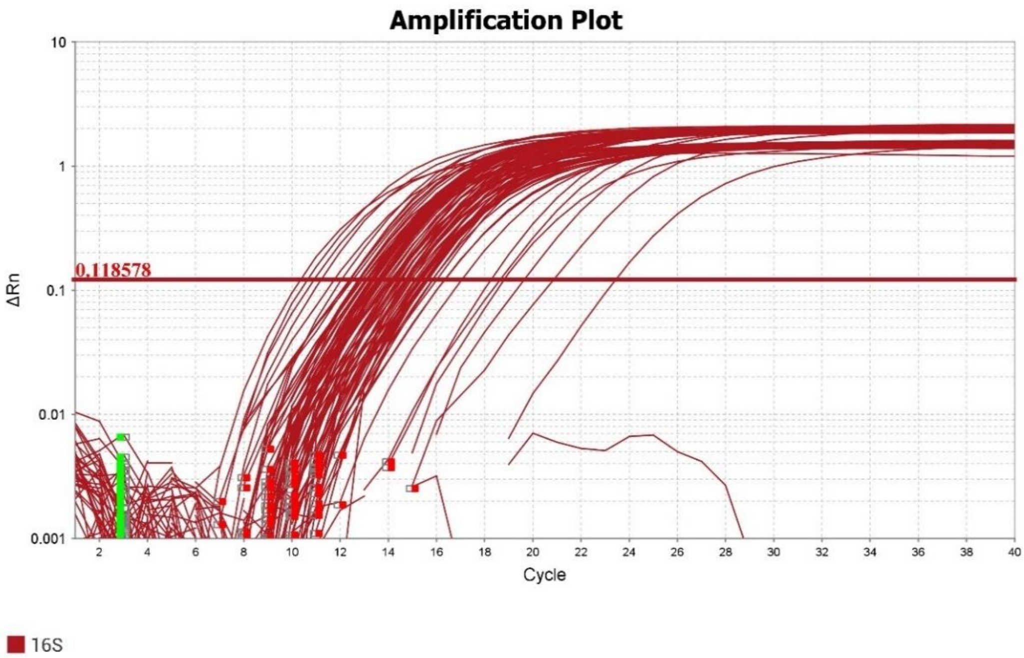

Figure 6. *16S rRNA PCR of 95 out of 100 pools. The plot above confirms that RNA extraction was successful.*

Animal health evaluation

A health evaluation was performed on 102 adult zebu cows, 21 (20.6%) males and 81 (79.4%) females, from which ticks were collected. Table 3 summarizes the intervals of observed parameters.

Table 3. *Range-span for checked health parameters (BCS=body condition score)*

Age (years)	Weight (kg)	BCS (1-5)	Health	Temperature (°C)	Tick count
1.5-8	75-395	2.5-3.5	16 different health statuses	37.3-39.6	10-150 ticks (one side)

The 16 health statuses were, animals in good health (n=45), swollen lymph nodes (n=25), dull hair coat (n=15), cracked skin (n=7), emaciation (n=6), dullness (n=4), inflamed perineal area (n=4), inflamed teats (n=2) tearing eyes (n=2), trouble breathing (n=1), corneal opacity (n=1), diarrhea (n=1), pus discharge scrotum (n=1), lameness (n=1), pot belly (n=1) and protruding pin bones (n=1).

In the Rupa sub-county 34/50 (68%) of the cattle were in good health compared to 11/51 (21.6%) in the Nadunget sub-county. One cow in Nadunget was not examined.

Of all the cattle 56/101 (55.4%) had one or more clinical signs, the rest were in good health.

Questionnaires

There were in total 50 participants, all cattle herd owners, 48 males (96%) and 2 females (4%). Their age ranged between approximately 20-80 years (not all knew their exact age) with an approximate average of 47 years. All of the interviewed cattle herd owners, had worked their whole life with cattle. Half of the participants were from Nadunget and the other half from Rupa.

Tick recognition

Looking at a colored picture of male and female *H. rufipes*, 44/50 (88%) of responders could recognize it as an "*Emadang*" which means tick in Karamojong or called it some other of the tick species they knew. The other six (12%), called it a cockroach, spider, scorpion or did simply not know.

Of the cattle herd owners, 94% (n=47) used color to differentiate different ticks. Pattern was used by 86% (n=43) and 48% (n=24) used size as a method. All three, color, pattern and size were mentioned by 38% (n=19) when asked how they distinguish different ticks.

In table 4 and 5 is a summary of what names were given for ticks shown on black and white and when shown in real life after being picked by the animals. Below is a list of the different names of ticks that were given during the interviews:

- Apinach/Epupa/Blue tick = *Boophilus* spp.
- Lethe/Nethe/Natheyo = Tick with striped legs (*Amblyomma* spp.)
- Lokomai = Spotted tick (*Amblyomma* spp.)
- Nagrakajen = Tick with striped legs (*Hyalomma* spp.)
- Najeje/red tick = Small tick (*Rhipicephalus* spp.)
- Nalebu = Big tick, any tick that was engorged

Table 4. *Summary of what the participants called the ticks shown on black and white pictures. To the left Karamojong names and on the top the ticks shown. The correct names are written in green. Note: when total is more than 100% it is because two names was given for the same tick*

	***Amblyomma* spp. m/f**	***R. Boophilus* spp.**	***Hyalomma* spp.**	***Rhipicephalus* spp.**
Did not know	22% (n=11)	38% (n=19)	30% (n=15)	32% (n=16)
Apinach/Epupa	0	2% (n=1)	4% (n=2)	2% (n=1)
Lethe/Nethe/Natheyo	14% (n=7)	8% (n=4)	18% (n=9)	8% (n=4)
Lokomai	44% (n=22)	18% (n=9)	14% (n=7)	18% (n=9)
Nagrakajen	4% (n=2)	6% (n=3)	4% (n=2)	2% (n=1)
Najeje	8% (n=4)	10% (n=5)	8% (n=4)	18% (n=9)
Nalebu	8% (n=6)	10% (n=5)	14% (n=7)	14% (n=7)
Other	2% (n=1)	8% (n=4)	6% (n=3)	6% (n=3)

Table 5. *Summary of what the participants called the ticks picked from the cattle. To the left Karamojong names and on the top the different ticks shown. The correct names are written in green. Note: percentage is of the amount of the ticks shown, and for a few cases the same tick was given two names*

	***Amblyomma* spp. m**	***Amblyomma* spp. f**	***R.Boophilus* spp.**	***Hyalomma* spp.**	***Rhipicephalus* spp.**
Did not know	0 (n=0/47)	0 (n=0/36)	n=0/14	0 (n=0/50)	2% (n=1/50)
Apinach/Epupa	0	0	71%	0	2% (n=1)
Lethe/Nethe/Nathe	30%	11% (n=4)	0	6% (n=3)	0
Lokomai	66%	31%	0	6% (n=3)	0
Nagrakajen	0	14% (n=5)	0	34%(n=17	0
Najeje	2% (n=1)	3% (n=1)	0	58%(n=29	88% (n=44)
Nalebu	0	44%	21% (n=3)	6% (n=2)	0
Other	2% (n=1)	3% (n=1)	7% (n=1)	2% (n=1)	8% (n=4)
Did not show tick	6% (3/50)	28%	72%	0	0

Animals and tick-borne diseases

A great variety of diseases were mentioned when asked which TBDs can be transmitted to animals, where the five most common ones were, anaplasmosis 72% (n=36/50), contagious bovine pleuro-pneumoniae (CBPP) 70%, mange 38%, ECF 36% and foot rot 20%. Other diseases were lumpy skin disease (18%), pox (16%), heartwater (6%), peste des petits ruminants (PPR) (6%), rinderpest (6%), anthrax (6%), babesiosis (4%), foot and mouth disease (4%), worms (2%), eye infection (2%), diarrhea (2%) and calf death (2%). Some clinical signs and other findings were also mentioned as fever (2%), bloody meat when open (2%), cysts when open (2%), get thin and die (2%), staggering (2%), killing udder (2%) and bleeding ear (2%).

All the participants answered that animals can get TBDs and 44% mentioned that both animals and humans may get sick of tick bites. See table 6 for the different named animals.

Table 6. *Summary of animal species susceptible to TBDs according to the cattle herd owners. Note: One person specifically pointed out that donkeys do not get sick from ticks*

Animal species sick by ticks	**Percentage**
Goats	84%
Cattle	76%
Calves	26%
Donkeys	12%
Dogs	12%
Sheep	10%
All animals	10%
Camels	8%
Big and small ruminants	4%
Wild animals	4%
Birds	2%

The interviewed persons were all worried about animals being sick and mentioned many diverse clinical signs when asked how they can see if an animal is sick in general. The most common one was standing hair, 42%. In total 38% mentioned that it affects the animals eating/grazing (stop eating, not eating with others, weight loss, etc.), 30% changes in movements (limping, staggering, uncoordinated movements, etc.) and 14% belly problems including changed feces and diarrhea. Other clinical signs were, wounds, shaking head, swelling, coughing, rapid breathing, anemia, blood in urine, getting thin, swollen lymph nodes, dry mouth, saliva, hair loss, tasteless meat, calves stop drinking milk, falling ears, running eyes and crying. The reasons why the cattle herd owners are worried are because cattle may die, they lose food (milk and meat), they live off animals, spending money on drugs and even that they may commit suicide if they lose animals.

To prevent cattle from getting bitten by ticks, 90% spray their animals with acaricides, 18% use both spraying and picking. Two responders (4%) point out that you cannot prevent animals from being bitten and one (2%) does not know how to prevent. The use of ivermectin is another way to prevent diseases according to 10% of the interviewed. Spraying outside and inside where they sleep, were mentioned as a method by 4%. One person mentioned that he calls the veterinarian to prevent and another person that they migrate to another place where there are no ticks.

Most participants, 89% think their method work to prevent ticks because they result in killing the ticks or remove them in other ways. One person (2%) says both yes and no, it kills the ticks, but it is slow. The last 9% does not think the method of spraying acaricides, drugs or picking is a working process to prevent ticks because it does not kill the ticks, they do not die fast enough, they are resistant, or ticks come back soon again.

If the animals do get bitten by ticks, 84% pick the ticks in different ways (with a thorn, hot ash, by hand) and most of the interviewed answered that they kill the ticks in a fire. Spraying together with picking is used by 34% of the participants when animals have ticks on them.

Humans and tick-borne diseases

All participants were worried about humans getting sick of tick bites, but 10% do not think that humans get bitten by ticks. A total of 9% replied that they do not think humans get sick from ticks. The most common symptoms affecting human after tick bites was swelling (68%), and ear problems (23%) and both clinical signs were associated with itching, fever and pus. The third most common negative effect of tick bites was pain (20%). Rashes, scars and diarrhea were other symptoms named to be caused by ticks. The reasons why the interviewed was worried is because people may die, it causes swelling and pain, they have to go the hospital, they may get infections and fever, who can take care of animals if shepherds die, it is better that animals die than human and because you cannot replace a person and humans are not easy to produce.

Two separate cases of severe hemorrhagic fevers including symptoms of melena (blood in feces), hematemesis (vomiting blood), epistaxis (nose bleeding) and ear bleeding were mentioned during the interviews. One case was a girl that survived and the other one a man that died.

For humans, 24% of the cattle herd owners answered that you cannot prevent humans from being bitten by ticks, and an additional 12% did not know how to prevent. Spraying animals and surroundings was used by 14% and good hygiene including bathing, sweeping, cleaning themselves was used by 12%. Checking themselves for ticks was used by 8% as a way of prevention. Monitoring the children and burning bushes were two other methods named by the participants. All of the interviewed persons believed their method of prevention works.

In case of tick bites, 88% said that they pick the ticks, using the same principle mentioned for animals above. Going to the hospital was another way used by 12% of the participants. One person uses cattle urine for three weeks, another person smear cream on, and one uses a drop of acaricide to handle tick bites.

Future

When it comes to prevention and handling of ticks in the future, as many as 94% said they need more drugs and better acaricides. Almost half, 42% thought that the government should help them, and 12% replied that there should be dip stations where they can take their cattle.

DISCUSSION

CCHFV

During the interviews, two cases of severe hemorrhagic symptoms were described. One was an adult person that died of hemorrhagic symptoms including melena, hematemesis, epistaxis and ear bleeding and the second was a girl with similar symptoms, but who survived. These findings suggest that the disease of CCHF might exist in the district, even though other hemorrhagic fevers such as Rift valley fever and Marburg cannot be ruled out. In total, 474 of the collected *Hyalomma* spp. ticks were screened for CCHFV RNA using qRT-PCR. Whether the ticks were collected from goats, cattle or the environment or whether they were of sub-species *H. rufipes* or *H. truncatum,* the results were all negative. One reason for not finding CCHFV could be because the sample size of 474 *Hyalomma* spp. ticks could have been too small to find the RNA of the CCHFV. In comparison, prevalence of tick-borne encephalitis virus (TBEV) in *Ixodes ricinus* ticks in Sweden was found to be 0.10-0.42%. This means that as low as only one tick out of thousand were positive in one of the pools (Brinkley *et al.*, 2008). Another notion is that only 19 of all the ticks collected belong to *H. rufipes,* the most probable host of CCHFV.

In the non-published study made by Balinandi *et al*., 2017, they found seroprevalence of antibodies towards CCHFV in serum samples taken from cattle in the same area, Moroto district, in both Rupa and Nadunget sub-counties suggesting that CCHFV circulates in the area, but as they did not collect and analyze ticks it is possible that other tick species than the *Hyalomma* spp. are vectors of the virus in this region. As written in the literature review, according to *Hoogstraal,* there are various ticks transmitting CCHFV including *A. variegatum* and *R. (B.) decoloratus* (Hoogstraal, 1979). In the study made by Balinandi *et al.*, they found prevalence of CCHFV in a *R. (B.) decoloratus* in Uganda (Balinandi *et al*., 2018*).* In the herein presented study neither the *Rhipicephalus* spp. (including *Boophilus)* nor the *Amblyomma* spp. were analyzed microscopically, but both *R. (B.) decoloratus* and *A. variegatum* have previously been found in Moroto district in 2015 (Byaruhanga *et al*., 2015) and by unpublished data from Balinandi *et al.* in 2017. Thus, it would be very interesting to check for prevalence of CCHFV in these other species.

Tick recognition

The general recognition of tick species was quite poor by looking at the black and white pictures amongst the Karamojong cattle owners. When shown pictures of the different species in black and white around 30% said straight away that they did not know the various species, and the correct given name for the ticks ranged from 2-18%, except for the *Amblyomma* spp. where more than half (58%) gave the correct name. When shown the ticks after being picked from cattle, the identification rate augmented vigorously compared to when shown in black and white pictures. The *Rhipicephalus* spp. was pointed out correctly by 88%, and the *R. (Boophilus)* spp. by 78%. The participants could also identify 34% of the *Hyalomma* spp., 96% of the male *Amblyomma* spp. but only 42% of the female *Amblyomma* spp. Then again, 44% of the female *Amblyomma* spp. and 21% of the *R. (Boophilus)* spp. were named *Nalebu,* which translates to big tick, and especially the female *Amblyomma* spp. grow quite big when more or less engorged.

The *Amblyomma* spp. has a typical colorful scutum in a very specific pattern covering the entire dorsal parts of the males and the front dorsal part of the females (Ogo *et al*., 2017). The fact that the *Amblyomma* spp. was the most recognized tick translates well, because when asked about how they distinguish the different ticks from one another, as many as 86% said by looking at the pattern. The pattern of *Amblyomma* spp. typically stand out on the black and white pictures compared to the other ticks. Of all the ticks picked from cattle, the *Amblyomma* spp. was also the most common one. More than two thirds (68.8%) of all ticks were of the *Amblyomma* spp. where 42.2% were males, this could also be a factor of why they more easily identified it in black and white.

Nalebu was the name given for engorged ticks, or ticks that have sucked enough blood to get bigger and the name did not seem to differ from one species to another. This makes it harder to analyze how well the locals actually know their different tick species. Female *Amblyomma* spp. was the tick that most people called *Nalebu*. Together with the other local names for *Amblyomma* spp. 86% in total could distinguish the females. A reasonably high number compared to the *Hyalomma* spp. (40% including *Nalebu*) that are similar in size and color but lacking the colored scutum.

The *Rhipicephalus* spp. is classified as a red-brown tick with a moderate size (Matthysse & Colbo, 1987), even though it is relatively small compared to the *Hyalomma* spp. and the *Amblyomma* spp. The identification percentage of the *Rhipicephalus* spp. was only 18% in black and white but a lot higher when seen for real (88%). Even though the sizes were marked with numbers of millimeters on the black and white picture, the presented drawings of ticks were similar in size by looking at them. As the North-Eastern part of Uganda, Karamoja, including Moroto district is the poorest part of Uganda (Uganda Bureau of Statistics, UNICEF & the World Bank, 2018), and also have the lowest rate of literacy (20%) (Okech, 2005), it could explain why the local cattle owners were struggling to identify the *Rhipicephalus* spp. by their size out of the drawings, but more easily in real. Obviously, color is another aspect to account for and 94% mentioned it as a mean to distinguish ticks from one another. The red-brownish legs are very typical, and definitely stand out compared to both the *Hyalomma* spp. and the *Amblyomma* spp. which both have slender legs with pale rings (Walker *et al.*, 2014). This is another factor which may explain why the *Rhipicephalus* spp. had a much higher percentage of identification in real life compared to black and white pictures.

Just as for the *Rhipicephalus* spp., the *Boophilus* spp. (included in the *Rhipicephalus* spp. but differentiated here for the ease of the study), also stand out when seen live. The shape for female ticks is elongated and *R. (B.) decoloratus* has a typical white-blue color and the size is smaller than *Hyalomma* spp. and *Amlyomma* spp. (Walker *et al*., 2014). On the black and white picture, it looks very similar to the other *Rhipicephalus* tick which may explain why the pastoralists were struggling to recognize it. Even so, both the *Rhipicephalus* spp. and the *Boophilus spp.* were both poorly identified in black and white. When presented in real though, the *Boophilus* spp. also had a relatively high identification percentage (71%), especially if the ones calling it *Nalebu* (21%) is included in the numbers. The shape, seen more clearly live and the typical colors are two factors that most likely aid in the identification.

The last species of ticks, *Hyalomma* spp. had a low rate of identification both in black and white and when shown live. For some reason, half of the participants called it surprisingly the same name as the *Rhipicephalus* spp. although they are different in size, color and the pattern of legs.

Amblyomma spp. has a similar pattern of the legs and are dark in color as well, but the dorsal pattern on the scutum of the *Amblyomma* spp. seem to stand out enough to not mistake it for the *Hyalomma* spp. The study was done while trying specifically to find *Hyalomma* spp. and despite that, the total amount of *Hyalomma* spp. among the collected ticks was only 7%. This could be the reason why the local Karamojongs are struggling to identify those ticks as they are in low numbers compared to the other species.

Finally, it is hard to draw strict conclusions of the names, and which ticks they represent as the people of Karamoja live remote and in small villages, where names probably get passed on from one another. Some of the same tick species might be called different names in different villages. An example of this is the *Apinach* and *Epupa*, where both seem to be meaning *Boophilus* spp.

Animals and tick-borne diseases

All participants were worried about their animals being sick from ticks, and 98% of all answered that both goats and cattle (cows + calves) are susceptible to diseases. This means that they are well aware of the problems tick may cause to their animals. Other animals were also mentioned as possible targets affected of ticks. Camels, dogs, donkeys, wildlife and even birds were all named. In total, the participants have relatively good knowledge of all the different diseases that may affect animals. How the diseases transmit to animals, seem not to be as clear to the interviewed. Only one person had good knowledge, and answered ECF, babesiosis, heartwater and anaplasmosis, which as written in the literature study, are all diseases spread by ticks. Most of the other Karamojongs were able to name different diseases they knew, but without seemingly knowing how they transmit. For example, anthrax, rinderpest and CCBP were all mentioned and the latter by as many as 70% of the persons even though it is caused by *Mycoplasma mycoides* and spread through direct contact between animals and not by ticks (Mariner *et al*., 2006). To a certain extent the participants seem to know what clinical signs to look for when an animal is ill, as they mentioned the classical signs of not eating, behavioral changes (not grazing with others), affected skin/fur and problems with movement. The importance of having healthy animals was demonstrated when a handful persons said that they might commit suicide if they lose their animals or that they have no other ways to live and feed themselves if their animals die.

Acaricides was named as the most common way to keep the ticks away and used by 90%. Which acaricides, how often and how effective their use is, was not clear. One reason to question the effectiveness and use of acaricides is because on average, 60 ticks were picked from each cattle and when counting the amount of ticks, they had on average 51.3 ticks per side which means at least 102.6 ticks in total. In another study made in Uganda where they compared dipping strategies, twice a week, once a month and not at all, the amount of ticks found were only 4.6 ticks when dipped twice a week, compared to around 60 for both dipping once a month and not at all (Okello-Onena, *et al*., 1999). Another study, from Patiko, in northern Uganda, with a monthly picking over an 18-month period, without using any form of prevention, found an average of around 300 ticks on each cattle (Kaiser *et al*., 1991). Of course, different climates, seasonal periods and areas could affect the amount of ticks but having over hundred ticks per animal is a lot if using proper functional acaricides. Then again, this is a very poor region, with few veterinarians and places where they sell drugs, which could explain why the use of acaricides is far from optimal. A few participants of this study also mentioned that they spray acaricides on the ground, where they sleep, and in the ear to remove ticks. This shows an apparent lack of understanding of the risks and how to use these agents. It has been proven that spraying acaricides in the environment can reduce the amount of ticks in that area, but it does not restrict the risk of being sick of ticks as they can get diseases from ticks from the surroundings where they have not sprayed (Hinckley *et al*., 2016). In developing countries acute pesticide poisoning is a serious problem and caused mainly by organophosphorus compounds. One of these compounds, chlorfenvinphos, has proven to be toxic by inhalation, ingestion and skin contact and cause all from mild to severe intoxication, including symptoms as nausea, headaches and vomiting (De Meneghi *et al*., 2016). Pesticides might not kill living organisms straight away, but instead cause chronic effects on physiology and reproduction, and cause resistance in microorganisms (Kunz & Kemp, 1994) and might therefore cause damage to the local flora and fauna.

An interesting aspect is that 89% think their method function as a way to prevent animals from being bitten by ticks. And as 90% are spraying, it might be working during the times acaricides are used, but that they are not used accordingly to the standards to get rid of ticks. In a study from 2016, they found that *Rhipicephalus* spp. were resistant to synthetic pyrethroids in 90% (27/30) of farms and a multi-acaricide resistance was seen in 55.2% (16/29) of farms, showed that acaricide resistance is widespread in Uganda. Super resistant ticks with 0% mortality was also found against two times discriminating dose of cypermethrin and deltamethrin (Vudriko *et al*., 2016). No samples were taken from the Moroto district (Vudriko *et al.*, 2016) and more studies should be done in the area to get an overview of what acaricides they use, how they are used and if there is resistance to any of the acaricides used.

After being bitten by ticks, the preferred method to remove the ticks was by picking the ticks, with a thorn, hot ash or by hand and then most often killing the ticks over fire. Seeing how well and fast the young children picked ticks for this study, it does not come as a great surprise that the participants pick a lot of ticks in general. According to Garci-Alvarez *et al.* transmission of disease is not guaranteed if animals or humans are being bitten by a tick (Garcia-Alvarez *et al*., 2013). The problem though is that if the cattle get infested by many ticks, it augments the risks and after being bitten it might be too late to protect from disease. An example is for ECF where the means of protecting cattle from disease is by using acaricides to prevent them from being bitten and thus transmitting disease (Oura *et al*., 2007). Being infested by many ticks may also cause blood loss, which is another risk factor to considerate.

Other drugs, especially the use of ivermectin was mentioned as a mean both to prevent and control ticks. Ivermectin is an avermectin, part of the macrocyclic lactone class, and is used as a highly effective endectocide, killing both endo- and ectoparasites. A long-lasting formulation with 3.15% ivermectin is widely used in the Americas, especially against the *R. (B.) microplus* infestation in cattle (Nava *et al*., 2019). On this basis, there is nothing wrong in using ivermectin as a drug to control tick infestation on cattle. But once again, there is a lack of knowledge of which kind of ivermectin, and other drugs for that matter, that is used for the cattle in the fight against ticks. The extent to which the participants treat their animals is also unclear. There are also risks associated with using high levels of ivermectin in cattle such as resistant ticks and helminths (worms) developing resistance (Nava *et al*., 2019), which is yet another reason why more studies need to be done of how they prevent and treat the ticks in the area.

Of all the cattle examined, 55.4% were affected by at least one apparent clinical sign, which suggests that there is a health issue in the region. For some reason there was a great difference in the amount of healthy cattle in Nadunget (21.6%) compared to Rupa (68%) sub-county. The environmental factors are quite similar including seasonal variations and rainfall. Nadunget and Rupa have a different responsible veterinarian for each sub-county. In Nadunget there is a young veterinarian 25-30 years old working, whereas in Rupa, the veterinarian is older and more experienced. This is a possible factor explaining these differences, but there may as well be others. For example, different economic conditions that this study is not aware of. How much these health problems are related to ticks, or to other factors is beyond this study.

For future concerns, it becomes clear that the local people of Karamoja need help in their battle against ticks and TBDs on animals. Not only because of health issues and the apparent limited knowledge of correct drug use, but also because almost all (94%) of the interviewed herd owners state that they require more drugs and better acaricides. Another fact to prove this point is that 42% of cattle owners explicitly mentions that they need more help from the government, and 12% asks for dip stations. If dip stations are a solution or not is yet to see, but more information, help and knowledge is definitely needed for the animals in this resource limited area.

Humans and tick-borne diseases

All the interviewed persons were worried about being sick from ticks, but in the questionnaire only 44% mentioned that humans may get sick of tick bites. Some herd owners, one out of ten, do not think humans get bitten by ticks, and 9% do not believe ticks make humans sick. These statements are contradicting themselves and makes it obvious that the herd owners need more knowledge about the risks of ticks. It has been proven that educational programs have effect as a way of prevention where for example teaching on how to dress and/or how to behave can help to avoid getting bitten by ticks (Garcia-Alvarez *et al*., 2013). This will still not be easy, as the Karamojongs are a people with old traditions for example dressed and behave following old cultural ways.

According to the responders of questionnaire, swelling and ear problems together with pain were the most common symptoms associated with tick bites. The literature agrees and states that local allergic reactions which causes swelling and forming papules are the most common clinical associations with tick bites (Doggett, 2004). *R. appendiculatus*, also named the brown ear tick is a common tick in Eastern Africa, and an important vector of several diseases including ECF (Kanduma *et al*., 2016), and it is probably this tick the participants refers to when they report humans being bitten in the ears. During the study, a small *Rhipicephalus* spp. was actually caught moving on the back of the neck of one child, probably on its way to the predilection site of the ear. The mentioned symptoms do not come as a surprise as they are all obviously related to tick bites. More interestingly is that a few persons point infections, fever and having to go the hospital in relation to the tick bites, which proves that some of them know the potential harm and severity of TBDs.

One fourth of every participant (24%) did not think it is possible to prevent ticks from biting people which is six times higher than for the animals. This could probably be explained by the use of acaricides for the animals. By spraying the animals and the surroundings, 14% thought that it would kill the ticks enough to keep them away from humans, which as stated above works to get rid of ticks, but likely not causing less disease problems. Personal protection by tick-checks and repellent are good strategies to prevent humans from getting bitten (Hinckley *et al*., 2016), and these were also mentioned by the cattle herd owners.

Different notions of hygiene were also standing out as a method of not getting bitten by ticks, but there is no evidence in the literature if being clean actually works to prevent ticks from biting. Physical and mechanical control to create an unattractive environment for ticks can reduce their infestation (Garcia-Alvarez *et al*., 2013) and for that reason sweeping is a good mean to keep live ticks away, but also to keep rodents and other potential reservoirs of infectious diseases agents away (Garcia-Alvarez *et al*., 2013). Living in a clean environment gives a better overview and can identify possible threats and therefore, might also help in the prevention.

On this ground, more knowledge about TBDs should be taught to the local people of Karamoja, to get a better understanding on which diseases are transmitted how, and how to properly and most efficiently protect the animals and humans from getting bitten and sick from ticks.

CONCLUSION

No CCHFV RNA was found in the collected *Hyalomma* spp. and this study could not prove *Hyalomma* spp. to be the vector of CCHFV in the Moroto district. The results do not exclude the virus and the disease of CCHF to be in the region as seroprevalence has previously been found in serum samples of cattle from the area and two suspicious cases of hemorrhagic fevers were discovered during the interviews with the local cattle owners. It could be that the sample size of 474 ticks were too small to find CCHFV. More studies need to be done in the region, and specially to look if other species, for example *Rhipicephalus (Boophilus) decoloratus* or *Amblyomma variegatum* are possible local reservoirs of the virus.

The cattle owners were a lot better at identifying ticks shown in real life compared to looking at black and white pictures and had a good understanding that ticks cause different diseases even though they could not properly point out which diseases.

It is always complicated to draw strict conclusions out of interviews, but one thing that is certain is that the locals of Karamoja need help from authorities to get a better general knowledge about TBDs and especially how to prevent them. Not only do the Karamojongs need help, but they also want the local authorities to support them in their fight against TBDs. More studies need to be done about the use of acaricides in the area. The local herd owners also need proper education about TBDs and their consequences to for example reduce the risk of tick resistance by incorrect use of drugs, including acaricides.

POPULAR SCIENCE SUMMARY

In 2017, Balinandi *et al.*, found out that cattle in Moroto, a district in North-Eastern Uganda, Africa, had antibodies against a virus called Crimean-Congo Hemorrhagic Fever Virus (CCHFV) in their blood. The CCHFV doesn't usually cause disease in animals, but it can be transmitted to humans by ticks or by handling affected animal products, for example blood from animal products. In humans, the virus causes a potential deadly disease with the same name, Crimean-Congo Hemorrhagic Fever (CCHF) and can result in harmful symptoms including fever, nausea, headache and different blood loss symptoms (nose bleeding, vomiting blood and/or ear bleeding). The aim of this study is to find if ticks of the genus *Hyalomma* spp., are infected by the virus CCHFV in Moroto district, an area that prior to this study never had any report of human cases of CCHF. The *Hyalomma* ticks are by previous studies known to be the most common tick species to transmit this virus, even though other species, for example *Rhipicephalus (Boophilus) decoloratus* has been shown to also transmit the virus. The ticks that are transmitting the disease, and the disease itself are wide-spread and have been found in Africa, Asia and Europe.

Another aspect of this study is to understand what the local cattle owners know about different ticks, their diseases, how to prevent tick bites and finally how they want to manage ticks in the future. With the consent of the cattle owners, ticks were collected by hand or forceps mainly from cattle, but also from goats and the environment. At the same time as the ticks were collected, the cattle owners were interviewed using a structured questionnaire. The ticks were then taken to a laboratory, at the Uganda Virus Research Institute (UVRI), in Entebbe. At the UVRI, the various ticks were firstly identified by looking at their appearance by the naked eye. The ticks that were identified as *Hyalomma* were furthermore divided into subspecies (spp.) by comparing certain features, for example punctuations (dots) on their backs, by microscopy. Finally, molecular studies, using a method called quantitative real-time polymerase chain reaction (qRT-PCR), were used to find out if any of these ticks had a unique genetical material, RNA (ribonucleic acid), belonging to the CCHFV.

No CCHFV RNA was found in the 474 *Hyalomma* spp. ticks in this study. It does not prove that the virus doesn't exist in the district of Moroto, but rather that there may be another tick species responsible for carrying and transmitting the virus in the area or that not enough ticks were tested. During the interviews, cattle owners in two separate villages mentioned that they have seen a person having the symptoms associated with CCHF as stated above. The two people mentioned were a man that died, and a young girl that survived. Based in this report, the suspicion that the CCHF disease exists in the area is strengthened, but other diseases with similar symptoms cannot be ruled out. The interviews showed that the cattle owners can reasonably well recognize all tick genera except the *Hyalomma* spp. by looking at them live. They know to some extent how to prevent cattle from being bitten, for example by using acaricides (drugs used to kill ticks), but the cattle still had a lot of ticks, which makes it questionable how well they know how to use these acaricides or if there is a problem with tick resistance. The participants of the study do quite well know which diseases animals can get sick of but show an apparent lack of understanding which ones are transmitted by ticks.

To conclude, more studies need to be done in the Moroto district to analyze how they use acaricides and in other ways prevent humans and cattle from being bitten by ticks, especially because they were all worried about diseases transmitted by ticks to humans and animals and that almost half of the interviewed explicitly said they need more help from government. New studies should be performed to find of out if there are tick species, other than *Hyalomma* spp. responsible for transmitting the CCHFV in the region.

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APPENDICES

Appendix A: Questionnaire

Location:

1. Sub-county:
2. Parish:
3. Village:
4. GPS co-ordinates:

Personal information:

5. How old are you?
6. Gender? M/Fe
7. Are you working with animals? Yes/No
8. How long have you worked with animals?

Ticks:

9. Do you recognize this insect? (Show 1. *Hyalomma* in color) Yes/No
10. What is the name of it? (Looking for tick in any language)

11. Do you know different types of ticks? Yes/No
12. If Yes, how do you distinguish different ticks? (Open q looking for color, size, animal they attack, other)

13. Can you identify any of these ticks? Show them four different black and white pictures of ticks, always in the same order. What's their names?

    2. (*Rhipicephalus (Boophilus) decoloratus*)
    3. (*Rhicicephalus* spp.)
    4. (*Amblyomma* spp.)
    5. (*Hyalomma* spp.)

Diseases

14. Do you know if ticks spread any diseases? Yes/No

15. If yes: Which diseases does it spread?

16. Who can get sick from ticks? (Looking for animals/humans as answer)

17. If yes animals: Which animals?
18. If yes, how does it show when animals get sick?

19. Has any person you know been bitten by a tick? Yes/No
20. If yes, did the human get sick? Yes/No
21. If yes, how did it show?

22. Have you seen a person with blood coming out from nose, mouth, ears and/or feces? (Looking for CCHFV, hemorrhagic diseases)? Yes/No

Prevention:

23. Are you worried about animals being sick from ticks? Yes/no
24. If yes, Why?
    If no, Why not?

25. What do you do to prevent animals from being bitten by ticks?

26. Does your method work? Yes/No
27. If yes, Why?
    If no, Why not?

28. What do you do if animals get bitten by ticks?

29. Are you worried about humans being sick from ticks? Yes/No
30. If yes, Why?
    If no, Why not?

31. What do you do to prevent humans from being bitten by ticks?

32. Does your method work? Yes/No
33. If yes, Why?
    If no, Why not?

34. What do you do if humans get bitten?

35. How would you want to manage ticks in the future?

Other:

36. Anything else you want to add?

37. Can you pick different species and tell us their names? (Show different picked ticks and ask for their names).

Appendix B: Consent Form

Consent Form

Study Title:

Prevalence of Crimean Congo Haemorrhagic Fever virus in Hyalomma ticks in Moroto, Uganda

Study Objectives

1. Investigate animal owner's/caretaker's knowledge about ticks and TBDs using a structured questionnaire
2. Investigate the prevalence of Crimean-Congo Haemorraghic Fever Virus in *Hyalomma* spp. ticks in Uganda

Consent:

I confirm that the purpose of the research, the study procedures, the possible risks and discomforts as well as potential benefits that I may experience have been explained to me. Alternatives to my participation in the study also have been discussed. I have read this consent form and my questions have been answered. My signature below indicates my willingness to participate in this study and have received a copy of this form.

Name... Sign.................................... Date..................................

For those unable to read and write

Name of witness.. Thumb print of participant

Sign..

Date..

Name of person obtaining consent Sign .... Date..................................

....

Appendix C: Tick 16s Real-Time RT-PCR Protocol - 2

MATERIALS:

Invitrogen's SuperScript III Platinum One-Step qRT-PCR Kit

- Primers and Probes
  - tick 16s 1F_A
  - tick 16s 1F_G
  - tick 16s 151R
  - tick 16s probe F51-77

MASTER MIX:

Reagent	Amount
2X PCR Master Mix	N x 12.5µl
RT/Platinum Taq Mix	N x 0.5µl
Forward primer IF_A (10µM stock)	N x 0.25µl
Forward primer IF_G (10uM stock)	N x 0.25µl
Reverse primer 151R (10µM stock)	N x 0.5µl
Probe F51-77 (5µM stock)	N x 0.5µl
*** Nuclease free water ***	N x 5.5 µl
Total volume	**N x 20.0µl**
Sample RNA	5 µl

CYCLING CONDITIONS:

	Conditions
Reverse transcription	50°C for 30 min
Taq inhibitor inactivation	95°C for 2 min
PCR amplification (40 cycles)	95°C for 15 sec 55°C for 1 min

Primers/Probes	Sequence (5'>3')
tick 16s 1F_A	ACT CTA GGG ATA ACA GCG T
tick 16s 1F_G	ACT CTA GGG ATG ACA GCG T
tick 16s 151R	GTC TGA ACT CAG ATC AAG TAG G
tick 16s probe F51-77	FAM - AGT TTG CGA CCT CGA TGT TGG ATT AGG - BHQ1