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Seroprevalence and risk factors for Rift Valley fever and Capripoxvirus in small ruminants in the border region of Tanzania - Zambia

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SUMMARY

Tanzania is a country where poverty is still high and many households are dependent on agriculture to support their families. Small ruminants, such as sheep and goats, make up an important part of agriculture; they are cheap to buy and can easily be sold or exchanged for the farmers. The animals therefore function as a living bank and should unforeseen expenses arise, the money can be made available by selling an animal. This means that the health of these animals is important socio-economically for the farmers.

Rift Valley fever (RVF) and sheep and goat pox (SGP) are two diseases that OIE have listed as notifiable. Rift Valley fever virus (RVFV) is an arbovirus transmitted by arthropod vectors such as mosquitoes. It is mainly infecting ruminants such as sheep, goats, cattle, buffaloes and camels, but is also a zoonotic disease and can infect humans. When domestic ruminants are infected, massive abortions can be seen in all stages in pregnant animals and a high fatality rate in young animals. Sheep and goat pox virus (SGPV) is a Capripoxvirus (CaPV) that belongs to *Capripoxvirus* genus. The virus is mainly transmitted through direct contact with infected animals, but indirect transmission through environment, or mechanical, through biting vectors, is also possible. Animals infected with SGPV show clinical signs of fever, loss of appetite, increased salivation and ocular and nasal discharge. After a few days, papules appear in the skin and on mucous membranes, even inside the body, which can cause serious and fatal complications. Young animals suffer more from the disease and the case fatality rate can be high. For farmers in rural communities, both diseases can have significant negative socio-economic impact, due to the loss of production and animals. The gender-equality between men and women may also be affected since women often are the main caretaker of the livestock.

This master thesis was performed as a Minor Field Study (MFS) that investigated the seroprevalence of RVF and SGP in Tanzania, in the two districts Momba and Tunduma close to the border of Zambia. The aim was to evaluate the seroprevalence in sheep and goats to understand the epidemiology of these diseases in the southwestern part of Tanzania and also investigate associated risk factors. Of the samples collected, 484 were from goats and 7 from sheep. Totally 16 of 491 analyzed samples were seropositive for RVFV, giving a seroprevalence of 3.3% on an individual level. All seropositive animals were goats, 93.8% females and 6.2% males. In total 31.7% (13/41) of the villages had seropositive animals, with a seroprevalence within the villages ranging up to 25%. The majority of the farmers reported that they utilized communal grazing system for their animals, where the majority of sheep and goats were reported to have daily contact with other domestic livestock. Only few sheep and goats had contact with wild ruminants. In this study, farmers buying their animals or had farmers in the same village buying their animals from markets, had significantly more seropositive animals

In this study only a single animal was seropositive for CaPV, a female goat belonging to the Momba district.

CONTENT

INTRODUCTION

Aim

The purpose of this study was to investigate the epidemiology of Rift Valley fever and Capripox in the southern part of Tanzania, close to the border of Zambia. To achieve the aim of the study, blood was collected from goats and sheep in two separate districts, Tunduma and Momba. Also, all farmers were asked questions (appendix 1) from a questionnaire regarding associated risk factors. Serum was analyzed by ELISA to investigate the seroprevalence of the selected infectious diseases. This master project was managed through close collaboration with scientists in Tanzania (Sokoine University of Agriculture) and is a smaller part of a PhD project with the aim to investigate the following infectious diseases; Rift Valley fever, capripox, peste des petits ruminants and foot and mouth disease and analyzing the risk factors for spread of these diseases in both Tanzania and Zambia.

LITTERATURE REVIEW

Tanzania

The country Tanzania is located in East Africa just below the equator with a total area of 947 303 km² (Central Intelligence Agency, 2018; Nationalencylopedin, 2018; Utrikesdepar-tementets landguide, 2018). The United Republic of Tanzania is made up by the mainland of Tanzania and the island of Zanzibar and is bordering eight countries; Zambia, Mozambique, Rwanda, Burundi, Congo, Uganda, Malawi and Kenya. Zanzibar has its own parliament and serves as a self-governing region. Tanzania consists of 31 regions governed by a president. The capital is Dodoma, located centrally in the country, but previously it was Dar es Salaam. Dar es Salaam is still the most important trading city in Tanzania, located by the Indian Ocean.

Ethnic diversity in the country is great and consists of around 125 different ethnical groups, and more than 100 languages are spoken within Tanzania (Central Intelligence Agency, 2018; Nationalencylopedin, 2018; Utrikesdepartementets landguide, 2018). Swahili is a Bantu language, which is one of four language families in Africa, and among the most common used in lower courts and primary school. English is introduced in secondary school and used in higher education and in higher courts.

Over the year, the country has two different rain seasons, one longer that occurs from March to May and one shorter rain season between October and January (Utrikesdepartementets landguide, 2018). During both rain seasons, the rainfall ranges between 750-1400 mm and spatial distribution varies over the country, but a greater amount of rain is seen closer to large water such as lakes or oceans (Hamisi, 2013). The dry season is mainly during the months from July to September.

Tanzania is administratively divided into 31 regions, and the regions are subdivided into 169 districts (Central Intelligence Agency, 2018; Nationalencyklopedin, 2018; Utrikesdepar-tementets landguide, 2018). The district is further divided into so called wards, and the ward

consists of different numbers of villages. The districts are the smallest administrative units with the responsibility for management of human and livestock diseases.

Animal and wildlife

Tanzania is famous for its beautiful nature and wildlife and almost a third of the landscape is national parks, game reserves, conservation areas and marine parks (Central Intelligence Agency, 2018; Nationalencylopedin, 2018; Utrikesdepartementets landguide, 2018). Tanzania's national parks contain around 20% of Africa's large mammal species, and the national parks attract tourists from all over the world, in particularly Serengeti National park in the north which is one of the main attractions. It is the second largest park and famous for its massive migration of wildebeests and zebra. Another park, the Ngorongoro Crater, is the largest caldera intact in the world. This caldera contains a rich wildlife and was in 1959 established as a conservation area by the United Nations Educational, Scientific and Cultural Organization (UNESCO). It is since appointed as a World Heritage site and through that legally protected by international treaties.

Agriculture

Human population in Tanzania is estimated to be around 56 million people and within the country poverty is still high (Central Intelligence Agency, 2018; Nationalencylopedin; Utrikesdepartementets landguide). Many people make a living through running their own farms, and small ruminants such as sheep and goats play an important role in the agriculture. They are often cheap to buy and easy to sell or trade for money, and these animals therefore have a big economic impact for the farmers. The animals can function as a living bank and when expenses arise the farmers can sell off some animals. The animals also contribute with products such as milk, wool, meat and skin, and are therefore important as food resources and for livelihood resilience for rural householders. The health management of small ruminants involves both genders and contributes to a more gender-equal society (Galie *et al*., 2017).

Momba and Tunduma

The two districts, Tunduma and Momba, belong to the Mbeya region (Wikipedia). In January 2016, Mbeya was divided, and the western half was renamed Songwe region. These districts are close to the border of Zambia. Tunduma town is on the border between Tanzania and Zambia and has border posts for the Tanzam Highway and Tazara railway, which link the two countries together. The 1,860 km long Tazara railway starts in Dar es Salaam in the east of Tanzania and reaches to Kapiri Mposhi in the central province of Zambia.

The border between Tanzania and Zambia is regularly crossed by civilians and vehicles (Africa business communities and Lusaka times). In recent time, a new border post has been established that has simplified the cross-border movement. It is possible that it will affect the animal trade and movements of animals between the countries.

Rift Valley fever

Etiology

Rift Valley fever (RVF) is caused by Rift Valley fever virus (RVFV) which belongs to the family *Bunyaviridae* and genus *Phlebovirus* (Epiwebb; Pepin *et al*., 2010). It has a spherical shape and is enveloped, just like other bunyaviruses and is about 90-110 mm in diameter (Pepin *et al*., 2010; Sherman *et al*., 2009). It consists of a single-stranded RNA with three segments of negative polarity: S (small), M (medium) and L (large) (Ikegami 2012). The virus has glycoprotein-peplomers, and these glycoproteins are important in the viral cycle and responsible for viral uptake of the virus in to cells (Pepin *et al*., 2010).

Rift Valley fever virus is sensitive to heat, desiccation, acid pH and disinfectiants (Quinn *et al*., 2011). Several of the viruses in the *Bunyaviridae* family are arboviruses, and thus transmitted through arthropods vectors, and this is also the case for RVFV.

The Rift Valley fever virus is primarily affecting and causing severe disease in ruminants such as sheep, goats, cattle and buffalos, but also camels have been found with the disease (WHO, 2018). Rift Valley fever virus can also infect humans which makes this a zoonotic disease. Amongst humans the virus causes acute clinical signs such as fever, headache, joint pain and abdominal pain. This therefore makes Rift Valley fever an important disease in a one health perspective.

Epidemiology

The first described outbreak of the disease was announced in July 1930 in Kenya by Daubney & Hudson (1931). The clinical manifestation was a high case fatality rate in new-born Merino lambs in a farm in the Rift Valley. The outbreak occurred when the farmer changed the lambing season from October and November to July and August. After the first outbreak, additional outbreaks were reported seventeen years later in 1947 (Sindato *et al*., 2014).

Rift Valley fever is endemic in sub-Saharan Africa (Rich & Wanyoike, 2010; Dar *et al*., 2013; Chevalier *et al*., 2005). A large outbreak was reported in Egypt 1977-1979, when 100 000 animals became ill and also a large number of people, around 200 000, were affected and approximately 600 of them died from the disease (Bowen, 2011). This was the first time the disease was discovered north of Sahara. In the year 1997 and 1998, the disease was found in Somalia, Kenya and Tanzania. Large amounts of animals like sheep, cattle, goats and camels became ill and many of them died during the outbreak (Bowen, 2011).

Rift Valley fever virus has a complex epidemiology, involving a wide range of factors where the key factors are considered to be rainfall and flooding, contact between animals, breeding sites of mosquitoes, movement and availability of livestock (Himeidan *et al*., 2014). These mentioned factors along with the complexity of different mosquito species, which can carry and spread the virus, make transmission possibly over big area of land. The presence of competent vectors in RVF-free countries with the ongoing climate change strongly suggests that the disease should be on the list of the most significant emerging viral threats to the public and

veterinary health (Pepin *et al*., 2010). The World Organization for Animal Health (OIE) has listed RVF as a notifiable disease (OIE, 2014).

Rift Valley fever in Tanzania

The livestock populations in Tanzania are the third largest on the African continent, with an estimated 25 million cattle, 16.7 million goats, 8 million sheep, 2.4 million pigs and 36 million chickens (United Republic of Tanzania ministry of livestock and fisheries development, 2015). The major part is located in the northern and central regions of the country (Chengula *et al.,* 2013).

In the country, a total of 10 RVF outbreaks have been reported in the past 80 years, the first in 1930 and then in 1947, 1957, 1960, 1963, 1968, 1977-1979, 1989, 1997-1998 and 2006-2007 (Sindato *et al*., 2014). Sindato and others conducted a retrospective study based on disease reported data on district and village level to investigate the spatial and temporal pattern of the RVFV outbreaks. Generally, the outbreaks occur between the months of December to June, the virus has been seen spreading southward, from Ngorongoro district in the north to Ulanga district in the south. In total, 39.2% of all districts in Tanzania have suffered from outbreaks varying in size. In Tanzania, the epidemiological features seem to be the same as in other countries. What makes Tanzania unique is that it is the only country with two branches of the Great Rift Valley ecosystem, the eastern and western, and these ecosystems are associated with RVFV (Sindato *et al*., 2014). These branches involve the whole country, starting in the north where it traverses from Kenya and carry out through the country to the southern parts.

Spatial investigations in Tanzania show that the northern part of the country appears to be the starting point for all outbreaks (Sindato *et al*., 2014). It is assumed to be an initial amplification site for the virus, and leading to spreading and progressive infiltration of RVFV to southern parts of Tanzania. The mechanism of spatial spreading is still unknown, but the authors propose the possibility of passive and active movements of mosquitoes and uncontrolled movement of livestock within the country. The authors conclude that the outbreaks of RVF in Tanzania seem to be heterogeneously distributed and the transmission of the virus seems to vary between areas and during seasons. That suggestion is consistent with the findings in other studies where seropositive animals were found during an inter-epidemic period in Morogoro and Arusha regions seven years after a major outbreak (Wensman *et al*., 2015). The authors concluded that it is an indication for the virus still circulating in low numbers in Tanzania. Furthermore, the authors take into consideration that the farmers in rural Tanzania is poorly prepared when the next outbreak arise due to no vaccinations against the disease are implemented.

In the beginning of the early outbreaks during the 1900s, Tanzania had a poor awareness of the disease, and inefficient recording systems and lack of capacity for diagnostics likely contributed to a low number of reports (Sindato *et al*., 2014). After 1978, RVF was listed as a notifiable disease by OIE and after that, monitoring and diagnostics have been improved. This has suggested caused the number of reported cases to increase.

About 12-13 years ago, in 2006-2007, the latest major outbreak was reported in Tanzania. This outbreak started in late December of 2006, and in January 2007 local veterinarians in the Arusha area started to report cases of massive abortions and deaths that could be caused by RVF (Sindato *et al*., 2014; Chengula *et al*., 2013). After the first report, additional reports came from Manyara, Kilimanjaro, Dodoma, Tanga, Iringa and Morogoro regions. The outbreak ended in July 2007 and had at that point affected 10 of 21 regions in the country and on a district level 45 out of 120 (Chengula *et al*., 2013; Sindato *et al*., 2010).

During the inter-epidemic periods, since the major outbreak in 2006-2007, studies detecting antibodies in both adult and young animals has been conducted in different parts of Tanzania (Wensman *et al*., 2015; Sumaye *et al*., 2013). Detections of these antibodies has also been in regions with no previous history of RVF outbreaks. This important finding shows that RVFV is endemic and circulating in low levels, within the country.

Rift Valley fever in Zambia

The first outbreak of RVF in Zambia was reported in 1974 and was located in the central and southern parts of the country, but there were also reports from the northern part belonging to the Copperbelt province (Hussein *et al*., 1987). Rift Valley fever is considered to be endemic in Zambia but for the last two decades no cases has been reported (OIE, 2014). During 1982-1986, a study was carried out in a sentinel herd with indigenous breeds in Mumbwa, and a low level of seasonal activity (3-8%) were found (Davies *et al*., 1992). Although low level of activity of the virus has been documented, no RVF-associated abortions or deaths have been observed (Rostal *et al.*, 2010; Davies *et al*., 1992). In 2012, a review demonstrated that Zambia's environment is beneficial for the virus, and thereby possessing a threat for the livestock producing farmers (Dautu *et al*., 2012). Little research has been carried out to study the presence of RVFV amongst humans, livestock and wildlife in Zambia, but OIE data between 2005-2010 show that most countries bordering to Zambia have reported cases of RVF. The studies conducted have been during outbreaks and in high risk areas and therefore information gaps exist (Dautu *et al*., 2012). Knowledge about different types of strains of RVFV in Zambia is also missing.

Transmission

Rift Valley fever was first suspected to be spread indirectly, because no natural spreading between sheep in the laboratory was observed (Daubney and Hudson, 1931). They later identified the vector *Taeniorhynchus brevipalpis* to carry RVFV. Linthicum *et al*., 1985 identified the virus in a variety of the *Diptera* species, and in *Aedes mcintoshi* the virus was present in both adults and offspring. They suspected a transovarian transmission, i.e. when the female mosquito directly passes virus on to her offspring, which later has been confirmed (WHO, 2018; Favier *et al*., 2006). Other genera of mosquitoes such as *Anopheles, Culex* and *Eretmapodites* can transmit RVFV, but this is mainly during epizootic periods when the level of virus circulation is high (House *et al.,* 1992). Rift Valley fever virus has been isolated in about 40 different species of mosquitoes that belong to seven different families: *Aedes, Anopheles, Culex, Eretmapodites, Coquillettidia, Ochleratus* and *Mansonia* (EFSA, 2005).

Aedes mosquitoes function as a primary vector. The mosquitoes bite an infected animal tissue, feed on blood, and carry and spread the virus to susceptible animals, in particular ruminants (Bowen, 2011). Many animal hosts can be infected, for example sheep, cattle, goats, camels, buffaloes and others (WHO, 2018). Some studies have shown that sheep and cattle can be more susceptible to RVFV than goats (Bird *et al.*, 2008). After susceptible animals become infected it takes between 3 to 5 days for them to become highly viremic and then spreading through also secondary vectors is possible (Bowen, 2011). Additional mosquito species have been experimentally infected with RVFV, and may act as amplifying secondary vectors (EFSA, 2005).

Heavy rainfall has been proven associated with outbreaks of RVF in Africa (Pepin *et al*., 2010). Most of the *Aedes*-vectors belong to the family of flood mosquitoes which favor laying eggs in flooded grassland areas and elevated water tables (Davies *et al*., 1985). For several years, the virus can be dormant in the mosquito eggs. The eggs can also survive for many years in the soil in dried flood beddings, and when rain season starts the eggs hatch (Fontenille *et al*., 1998; Linthicum *et al*., 1985). Several other risk factors have also been associated with the occurrence of the virus, and these includes climatic conditions such as higher temperature, geographical features, vegetation cover, human activities and livestock trade in the country (WHO, 2018).

Rift Valley fever virus is not only transmitted through arthropod vectors but also through direct contact between susceptible hosts and infected animal tissues, body fluids and aborted materials (Pepin *et al*., 2010). Aborted materials, such as fetus and placenta, have been identified to contain large amounts of the virus.

In Zambia, no studies have been conducted regarding potential mosquito species that can spread the disease (Dautu *et al*., 2012). Different species of vectors have been observed to differ depending on region and season, within the country (WHO, 2018).

Potential European vectors

In Europe, there are potential vectors that should be able to carry and spread RVFV (Rolin *et al*., 2013). In order for the virus to be introduced into a new region, two factors are needed, optimal environmental conditions and introduction of virus are required for a period of time. The authors conclude that the conditions are still not optimal for the virus to be spread in to Europe, but the risk of the pathogen being sporadically introduced is likely to be relatively high (Rolin *et al*., 2013).

The European Food Safety Authority (EFSA) has highlighted the need of increased knowledge about which of the mosquito species in Europe that have the ability to carry and transmit the virus (Verteirt *et al*., 2013). In this study, periods of peaks of *Aedes* and *Culex* mosquito species in some areas could pose a risk, a great quantity of mosquitoes was mainly observed in the coastal areas in countries of the Mediterranean Basin.

Clinical manifestation

The virus primarily affects ruminants such as cattle, sheep, goats and buffaloes (FAO, 2003) and cause a diffuse clinical picture with nasal and ocular discharges, fever, colic, vomiting and hemorrhagic diarrhea (Ikegami, 2012; SVA, 2018). The incubation time is about 3 days (Bowen, 2011). The main clinical manifestation is epidemic abortions (so called abortion storms) in pregnant animals infected in all stages (Epiwebb, 2018). Rate of abortion can be as high as 90-100% in affected pregnant animals in all stages (OIE, 2018).

Rift Valley fever virus has been detected in several tissues and cells in the body (Pepin *et al*., 2010). After infection, a local replication occurs in the regional lymph node that leads to viremia with systemic spreading (Bowen, 2011). The systemic spreading is affecting internal organs, in particularly the liver where necrosis can occur, but also the spleen can be affected. Viral replication has been seen in other tissues like adrenal glands, lung and kidney tissues. In some rare cases the virus can affect the brain and cause encephalitis. Animals often die because of necrosis in the liver, renal insufficiency and shock, frequently with severe hemorrhagic (Bowen 2011).

Out of all ruminants, it seems like sheep are affected most seriously by RVFV (Pepin *et al.*, 2010). But out of all ruminants that can be affected it is the young animals that are more susceptible to the virus (Epiwebb; SVA; Pepin *et al*., 2010). In young animals, sudden death can occur without any clinical signs, while in some cases a high fever develops and the animal dies after a day. This causes a high case fatality rate (Chengula *et al*., 2014), and in young animals younger than one week, the case fatality rate can be as high as 90% (Quinn *et al*., 2011).

Diseases like listeriosis, Q-fever and toxoplasmosis have similar clinical manifestation, but the abortion storms that occur when RVFV infects ruminants make other differential diagnosis more unlikely (Pepin *et al*., 2010).

Rift Valley fever virus can infect humans and therefore RVF is also a zoonotic disease (Reed *et al*., 2012). The incubation time is from 3 to up to 12 days before symptoms first starts to appear. In humans, RVFV causes symptoms of acute fever, headache and pain in muscles and joints (Ikegami, 2013). The symptoms often decline after 4-7 days, when the viremia declines and antibodies start to develop (OIE, 2018). In some humans, complications can occur such as bleedings, liver failure and encephalitis (WHO, 2018). The case fatality rate is 1%. Transmission of RVFV can occur through arthropods vectors but also from organs and body fluids of infected animals to humans (OIE, 2018). Occupational groups, such as farmers, slaughterhouse workers and veterinarians, are therefore at higher risk of infection (WHO, 2018). In previous studies in the Mbeya regions, that include Momba and Tunduma district, a seroprevalence of 5.2% has been observed in humans (Heinrich *et al*., 2012).

Diagnostics

International organizations such as OIE have expressed demands for the development of high quality and safe diagnostic tests for RVFV. Working with RVFV pose some problems, it

requires high biosecurity and biocontainment safe facilities when handling (Pepin *et al*., 2010). Rift Valley fever virus is considered to be a potential bioweapon.

Several kinds of diagnostic methods are available to diagnose RVF and different techniques are used, such as antigen detection, virus isolation, nucleic acid amplification techniques and detection of specific antibodies (Pepin *et al*., 2010; OIE, 2009; Epiwebb, 2018; SVA, 2018). To detect RVFV, amplification of RNA fragment can be done by a newer more efficient molecular diagnostic assay with reverse transcriptase polymerase chain reaction (RT-PCR) and reverse transcription loop-mediated isothermal amplification (RT LAMP) (OIE, 2018; Pepin *et al*., 2010). These processes can take a long time before providing results, but it has a high sensitivity for detecting the virus RNA.

Enzyme-linked immunosorbent assay (ELISA) is one of several serological methods, and the technique is used to identify RVFV specific antibodies (Pepin *et al*., 2010). Depending on how the ELISA is designed, it can detect IgG, IgM or total antibodies. The recommended ELISA is the one based on RVFV recombinant antigens (Pepin *et al*., 2010; Jansen van Vuren *et al*., 2007).

Vaccination

Outbreaks of RVF can be prevented through vaccination programs, where both modified live attenuated and inactivated virus vaccines can be used (WHO, 2018). The live attenuated vaccine only requires one dose for long-term immunity, but spontaneous abortion has been seen in pregnant animals (WHO, 2018). The inactivated virus vaccine requires multiple doses for immunity but no side effect with spontaneous abortion has been seen. Efficient and safe vaccines for both medical and veterinary use are, however, still lacking (Pepin *et al*., 2010). Recently, highly immunogenic vaccines have been developed, and they will probably replace live attenuated vaccines, but further evaluations are required to confirm safety and efficacy of this vaccine (Ikegami, 2017). Old vaccines, such as MP-12 vaccine, have been tested on pregnant ewes, they were subcutaneously vaccinated and all ewes delivered healthy lambs (Morill *et al*., 1987). Ikegami, 2017 suggests that further testing of vaccines, such as MP-12, would be an option when the market for RVF vaccines is small. To evaluate the MP-12 vaccine, in both humans and animals, would not require additional investment.

Sheep and goat pox

Etiology

Sheep and goat pox viruses (SGPV) are Capripoxviruses (CaPV), belonging to genus *Capripox*, sub-family *Chorodipoxvirinae* and family *Poxviridae*. Capripoxviruses are large, (170-260 nm in diameter), enveloped, and double-stranded DNA viruses (Carn 1993; Tulman *et al*., 2002; Buller *et al*., 2005). Sheep pox virus (SPPV), goat pox virus (GTPV) and lumpy skin disease virus (LSDV) are a series of CaPVs affecting domestic ruminants and causes various pox diseases. Genome sequences of SPPV, GTPV and LSDV show that they are highly similar with more than 96% of the nucleotides identical (Tulman *et al*., 2002). Sheep pox virus and GTPV appear to be specific for each host species, but recent isolates show the ability to infect both

hosts, and are therefore concluded as SGPV (Babiuk *et al*., 2009). The varieties of strains for SGPV are phylogenetically distinct from each other and are named based on the host species that it has been identified from (Kitching *et al*., 1989; Kitching 1986; Tulman *et al*., 2002). They cause different clinical diseases in either sheep or goats, and some strains are similarly pathogenic in both species (Babiuk *et al*., 2008). Sheep and goat pox virus is vulnerable to direct sunlight, but in wool and hair it can remain viable for up to three months (Epiwebb, 2018; OIE, 2018). In shaded dirty pens, SGPV can survive for up to six months.

The CaPVs are an economically important group of viruses because of their severe impact on deprived rural communities and small-scale farmers in endemic regions (FAO, 2013). World Organization for Animal Health classified SGP as a notifiable disease in 2014. Sheep and goat pox are among the most common diseases in sheep and goats entailing a huge economic loss for affected countries. For farmers, the production losses can be significant and affect their socio-economic standing (Yeruham *et al*., 2007). Sheep and goat pox is also limiting the international trade of animals and animal products (OIE, 2018).

Epidemiology

Sheep and goat pox viruses are spread over a variety of continents over the world. In the Middle East, SGPV is endemic in countries like Turkey, Iran, Syria and Iraq (OIE, 2014; Mangana *et al*., 2008). Sheep and goat pox viruses are mostly restricted to Asia and north of the equator in Africa.

In Europe, SGP is an exotic disease but several neighboring countries are endemically infected with SGP (Kitching, 2004). After the veterinary service failed in Syria, uncontrolled movements of livestock pose a major risk of spreading the diseases to Europe (FAO, 2013). Sporadic outbreaks have been reported in European countries, mostly in Greece (Mangana *et al*., 2007). In Greece, it is mainly sheep pox that has been reported, and during some outbreaks, like the one in 2007 when the case fatality rate was high, it was concluded that possibly the sheep pox virus was of a highly virulent strain or the hosts had a low immunity to the virus. The affected herds were mixed with both sheep and goats and serologically it was proven that antibodies to CaPV were present in goats, but no clinical signs were reported. The geographic position of Greece makes the country an important area for control of introduction of SGP to Europe, due to the fact that neighboring countries are enzootic (Mangana *et al*., 2007). Greece has applied stamping-out/non-vaccination policy since 1992 whenever there is an outbreak in the country.

EFSA evaluated the possible ways of SGP introduction into Europe in 2014, and the most important risk factors identified were movements of people and vehicles across the borders (EFSA, 2014). The movement can be immigrants passing through endemic areas carrying the virus over long distances, but also tourists, farmers, veterinarians and animal care workers can transmit the virus over the border when they are visiting animal facilities. EFSA also concluded that movement of infected animals over the border is assumed to be the most efficient way to introduce SGP to new areas but it is considered to be less important than movement of people and vehicles (EFSA, 2014).

Sheep and goat pox is endemic throughout the northern and central part of Africa (Carn, 1993), and many reports of SGP have also been from East African countries, namely Sudan and Kenya (Enan *et al*., 2013; Ahmed, 2012; Elshafie & Ali, 2008; Davies *et al*., 1985). Sheep and goat pox has recently been serologically proven to be present in Ethiopia and considered to be the most important disease in the country (Fentie *et al*., 2017).

Sheep and goat pox in Tanzania

In Tanzania, no reports of SGPV have been done until 2018, CaPV were detected during a massive outbreak of respiratory disease in 2016 in sheep and goats (Kgotlele *et al*., 2018). This outbreak was located to the Ngorongoro district in the north of Tanzania and confirmed occurrence of co-infection with pathogens that are associated with respiratory distress such as PPR.

Sheep and goat pox in Zambia

In Zambia, no reports have been made about presence of SGPV (OIE, 2018). Outbreaks of LSDV on the other hand have been reported within the country. Clinical signs of lumpy skin disease (LSD) were first described in Zambia in 1929, and were first believed to be a result of either poisoning or hypersensivity to insect bites (FAO, 2013). Thereafter, cases occurred between 1943 and 1945 in neighboring countries such as Botswana, Zimbabwe, Mozambique and in South Africa (Green, 1959; Von Backstrom, 1945).

Transmission

In natural infection, SGPV enters the respiratory tract through aerosols associated with close contact with infected animals (Radostits *et al*., 2006). Inhalation of aerosols to the respiratory tract is the primary transmission route of the virus (Kitching & Taylor 1985). Experimental studies of SGPV transmission have used intradermal inoculation or administration by mouth or nose (Bowed *et al*., 2008).

Sheep and goat pox viruses are extremely tolerant in the environment and can survive for a long time in shaded dirty pens (Epiwebb, 2018; OIE, 2018). Therefore, spread through indirect transmission is possible. Virus has also been found in urine and feces leading to contamination of the environment (Bowed *et al*., 2008). Wool of previously infected animals has also been identified as a risk factor for spreading the disease, because it can contain virus for a long time after the animals have recovered (EFSA, 2014). The virus is however sensitive to direct sunlight.

Different kinds of vectors have been shown to spread SGPV, such as biting flies (*Stomoxys calcitrans*) (Kitching *et al*., 1989; Mellor *et al*., 1987; Kitching & Mellor; 1986). In other studies, no virus transmission was observed with lice or fleas in sheep, even though virus was isolated from infected sheep (Kitching & Mellor, 1986). Despite this, some researchers suggest that mechanical transmission through biting insects might be more common than previously suspected (Bowed *et al*., 2008), because the skin of infected animals contains high titers of virus (Babiuk *et al*., 2009; Bowed *et al*., 2008).

In summary, transmission of SGPV occurs through different pathways and both by direct and indirect contact with oronasal secretions, aerosols, and respiratory droplets produced by acutely infected animals (Verma *et al*., 2011; Kitching & Taylor, 1985).

Clinical manifestation

Sheep and goat pox can affect animals in all age groups and causes severe pox diseases in small ruminants (Kitching 2004; Radostits *et al*., 2006; Epiwebb, 2018). Signs can be acute to mild and in some cases subclinical. The morbidity rate can in susceptible herds be up to 75-100% and the case fatality rate in flocks with young and old animals could be as high as 100%. In some flocks, the case fatality rate can also be 90%, due to the fact that animals simultaneously suffer from another viral condition, such as peste des petits ruminants (PPR) (Radostits *et al*., 2006). In Europe, the naive population of sheep and goats are more susceptible to SGP than those in African and Asia (EFSA, 2014) were SGP are endemic.

The incubation period is from 5 to 6 days up to 2 weeks (Bowed *et al*., 2008; Epiwebb, 2018; OIE, 2018). Sheep and goat pox has an acute progression with clinical signs such as swelling of the nostrils, nasal discharge with high viscosity, serous discharge from the eyes and marked depression (Radostits *et al*., 2006; Bowed *et al*., 2008; Epiwebb, 2018; OIE, 2018). These signs start together with pyrexia (about 40-42ºC), difficulties in breathing and loss of appetite. After a few days, lesions start to occur on un-wooled skin in the face, around the lips and on the eyelids, as well as in the mucosa of buccal, respiratory, digestive and uro-genital tracts. At first, the lesions arise as macules, and then develop into papules. The papules become ulcerated and necrotic and in the next 5-10 days scabs will forms. Lesions can cover the entire body but are visible in hairless parts of the skin, oral cavity and mammary glands. After the animals have been infected, it takes from 6 to up to 10 days before infected animals start secreting virus from the nose, conjunctiva and oral cavity. Virus occurrence was found to be correlated with the appearance of ulcerated lesions on mucosal surfaces (Bowed *et al*., 2008). These ulcerative lesions could be seen in mucous membranes in the mouth, nasal cavities and throughout the digestive and respiratory system. Those lesions can cause serious clinical signs because of complications due to secondary bacterial infections. Also, in infected animals involvement has been observed of the lymphoid tissues, liver and spleen with detection of virus. In adult animals, it is not common with the systemic complications seen in young animals, but abortion and secondary pneumonia can be observed and the lesions are primarily observed on the skin on the underside of the tail. Adult animals often recover after 3 to 4 weeks with permanent depressed scars. Shedding of virus has experimentally been observed to occur up to 41 days after inoculation for goats and 64 days for sheep from nasal discharge.

Diagnostics

World Organization for Animal Health (OIE) has categorized SPPV and GTPV as notifiable diseases, because of their potential for rapid spread and considerable economic impact. Therefore, diagnostic monitoring plays an important role to identify the virus spread to susceptible livestock.

Sheep and goat pox is a clinical diagnosis due to the characteristic clinical manifestations with lesions, species affected and post mortem findings (SVA,2018 & OIE, 2018). The clinical signs are similar to diseases such as foot and mouth disease (FMD), dermatophilosis/streptothricosis, photosensitization and PPR, and therefore it is necessary to use laboratory methods to confirm the diagnosis. The generic CaPV real-time polymerase chain reaction (PCR) is the gold standard and detects CaPV DNA, but does not differentiate between different virus species (EFSA, 2014). In a recent study, it has been suggested that PCR assay based on the RPO30 gene can be used to identify all CaPV infections (Mahmoud and Khafagi, 2016).

The test most used, after PCR, is serological test such as ELISA. The ELISA will not distinguish between different strains of SPPV, GTPV and LSDV, it will only detect the group of antibodies against CaPV. Together with PCR, ELISA is considered to be the most sensitive and specific diagnostic tests (EFSA, 2014).

To eradicate SGP the same strategy can be adopted that was followed in case of rinderpest (OIE, 2018). That includes serological surveillance and vaccination, with initial mass vaccination. For a country to be declared SGP-free it requires a period of ten years free from the disease.

Vaccine for SGP

For SGP there are a variety of live attenuated and inactivated CaPV vaccines. To protect against SGP it is possible to use a single strain of CaPV for both species, and this vaccine gives protection for all field strains of virus regardless of their geographical origin (Kitching *et al*., 1986; Kitching & Taylor, 1985). The inactivated vaccines only have a short-term immunity (OIE, 2018).

A new generation of vaccine is under development using CaPV genome as a vector for the genes of other pathogens such as peste des petits virus (PPRV) (Tuppurainen *et al*., 2014). The possibility with this vaccine is that it will provide protection against SPPV, SGPV, LSDV and PPRV. This recombinant vaccine is not commercially available yet.

MATERIAL AND METHODS

Study area and study design

This study was a cross-sectional serological survey that investigated the seroprevalence of RVF and SGP. The aim was to investigate the epidemiology of the diseases by detecting antibodies in serum collected from sheep and goats in villages. The study was conducted in the southern part of Tanzania in two districts, Tunduma and Momba (Fig. 1), close to the border of Zambia. These districts were selected from the Mbeya region due to the proximity to the Tazara railway and Tanzam highway, close to the Zambia border. In the Tunduma district, 8 villages were visited, and in the Momba district, 33 villages were visited. Villages included in the study were randomly selected from a list provided by a District Veterinary Officer (DVO) for Momba district, and a list provided by a District Livestock Officer (DLO) for Tunduma. For each

village, GPS-coordinates were recorded and before initiating blood sampling of the small ruminants, a written consent were signed by the farmer.

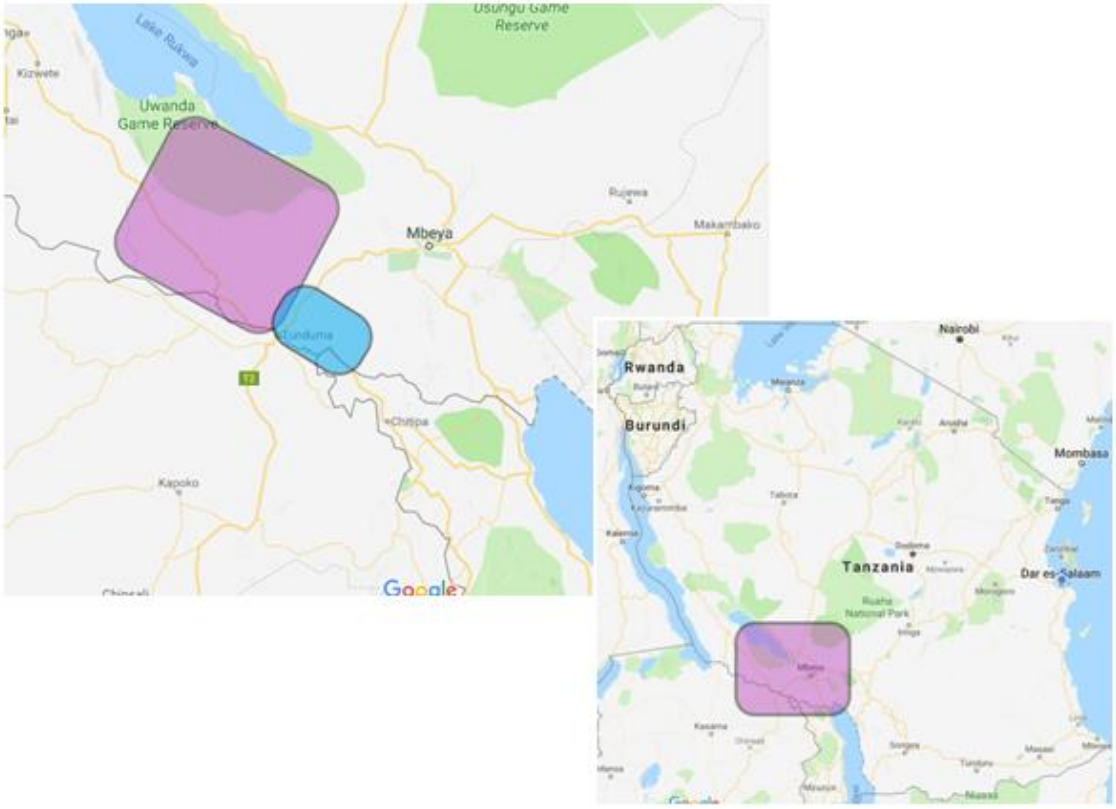

Fig 1. *The approximate locations of the two districts, Tunduma and Momba, in the southwestern part of Tanzania. Purple = Momba. Blue = Tunduma.*

From every village, four households were selected using snowball sampling method. The first step was to approach a farmer in the village, sampling his/her goats and then ask for the next farmer with sheep and/or goats. The criteria for applying the method following questions were asked to the farmer;

1. Do you know a livestock keeper with less than 5 sheep and/or goats?
2. Do you know a livestock keeper with 5-15 sheep and/or goats?
3. Do you know a livestock keeper with more than 15 sheep and/or goats?

However, these criteria were not always met due to some villages only having farms with small number of livestock. In these cases farmers were asked giving direction to just any farm in the village with sheep and/ or goats.

Animals and sampling

This study was approved by an ethical committee (ILRI-IREC 2018-04). The sample size was calculated according to a method described by Humphrey, Cameron & Gunn (2004) to estimate a true prevalence on animal-level and herd-level. For estimation, a prevalence of 50% was used for a maximum sample size, and the calculation was based on the ELISA that gave the highest sample size which were RVF competitive ELISA. For RVF competitive ELISA the sensitivity

is 0.91-1 and the specificity 1, the confidence interval was 0.95 used and precision 0.05. The sample size required was 461 blood samples, and therefore, it was decided to test 491 animals. Blood samples were collected during a single field trip during September 2018.

Sheep and goats were randomly selected for blood sample collection. At every farm, collections of blood samples were conducted from at least three sheep and/or goats. However, in some farms the households had less than three animals, in those cases all animals were selected and sampled.

Fig. 2. *Blood sampling during the fieldtrip in Momba district. Photo: Elsa Wilén.*

Collection of blood was obtained from the jugular vein on restrained animals using a syringe, vacutainer, and blood collection tubes (BD Vacutainer, Plymouth, UK) (Fig. 2). From each individual, one serum tube was collected, and individual data was collected about species, breed, gender, age, clinical signs at time of blood collection and any clinical signs observed in the last 12 months.

After sampling, the serum tubes were put in a waist bag that was carried throughout the village. The tubes where then placed in a cooling bag in the car and kept there until the end of the day. The serum was separated and placed in cryotubes the same day or the day after sampling. During the field trips the samples were stored in different freezers with a temperature around –9 ºC until transportation back to Morogoro.

Antibody detection

RVFV competitive ELISA

The test used in this study for detection of antibodies to RVFV was a competitive ELISA (ID Screen Rift Valley Fever Competition Multi Species, ID-vet, Grabels, France). This test detects antibodies that are directed to RVFV nucleoprotein in both serum and plasma. Detection of antibodies indicates exposure to RVFV by natural infection or vaccination. The competitive ELISA does not differentiate IgM from IgG antibodies. The sensitivity is 91-100% and the specificity is 100%, according to the manufacturer.

The ELISA was used according to the manufacturer's instructions and are briefly described below. First, 50 µL of dilution buffer was added to each well. After this, 50 µL of each sample were added to corresponding well except for the wells where 50 µL of the positive and negative control were added. The plate was covered and placed in a shake incubator (Micro Shake ELISA Plate Shaker) for 1 hour ± 4 min at a temperature of 37 ºC (± 2 ºC), to allow homogenization of the samples through vibrations throughout the incubation period. After incubation, the plate was washed 3 times with 300 µL of wash solution. Then 100 µL of conjugate was added in to each well. The plate was then covered and incubated for 30 ± 3 min at a temperature of 21 ºC (± 5ºC). After another wash step. 100 µL of substrate solution was added to each well. The plate was then covered and incubated 15 ± 2 min at a temperature of 21 ºC (± 5ºC) in the dark. After that 100 µL of stop solution was added in each well in the same order as substrate solution was added to stop the enzymatic reaction. Followed by plate reading at 450 nm with a Erba Lisa Scan II (Erba Mannheim). Calculations were made to check the validity of the positive and negative control. For a valid test the mean value of the negative test needed to be higher than 0.7 and quota of the positive control divided with the negative control should be lesser than 0.3. For each sample, the competition percentage value was calculated through dividing the sample optical density (OD) value with the OD value of the negative control, multiplied by 100. Samples with a competition percentage ≤ 40% were considered positive and a competition percentage ≥ 50% were considered to be negative, in between samples were considered to be doubtful.

Double antigen ELISA for Capripox

The test used in this study for detection of antibodies to CaPV was a double antigen ELISA (ID Screen Capripox Double Antigen Multi-species, ID-vet, Grabels, France). This test is designed to detect antibodies for CaPVs causing goat pox, sheep pox and lumpy skin disease. The diagnostic test can be used with serum or plasma from individuals of cattle, sheep, goat and other susceptible species. According to the manufacturer, it has been shown to have a specificity of >99.7% in CaPV free regions (ID-vet).

The ELISA was used according to the manufacturer's instructions and are briefly described below. First, 50 µL of dilution buffer was added to each microwell. After this, 50 µL of each sample was added to corresponding microwell, except for the wells where 50 µL of the positive and negative control were added. The plate was covered and incubated for 90 ± 9 min at a temperature of 21 ºC (± 5ºC). After incubation, the plate was washed 5 times with 300 µL of wash solution. Then 100 µL of conjugate was added in each microwell. The plate was then covered and incubated for 30 ± 3 min at a temperature of 21 ºC (± 5 ºC). After another wash step. 100 µL of substrate solution was added to each well and the plate covered and incubated for 15 ± 2 min at a temperature of 21 ºC (± 5 ºC) in the dark. After that 100 µL of stop solution was added in each microwell in the same order as substrate solution was added to stop the enzymatic reaction, followed by plate reading at 450 nm with a Erba Lisa Scan II (Erba Mannheim). Calculations were made to check the validity of the positive and negative control. For a valid test the mean value of the positive test needed to be higher than 0.350 and the ratio of the mean value of the positive and negative control to be higher than 3.

For each sample, calculations were made with the following formula:

$$\frac{\text{Sample value} - \text{mean value of negative control}}{\text{Mean value of positiv control} - \text{mean value of negative control}} x\ 100$$

Samples that were < 30% was considered negative and ≥ 30% were considered to be positive.

Questionnaire

In addition to blood sampling, a questionnaire in English was used to interview each farmer about the perceived socio-economic impact of infectious diseases. A PhD student from the Sokoine University of Agriculture conducted the questionnaire in Swahili since many farmers only spoke Swahili. The questionnaire contained multiple questions about management procedures, health status, contact with other herds and wildlife etc (Appendix 1).

Statistical analyses

The statistical analyses from the results of the serology were processed in Excel and the Chi-square calculations by Social Science Statistic (Stangroom, 2018). It was used to compare seropositivity between goats and risk factors. A confidence interval of 95% was used in this study.

RESULTS

Study area and animal and sampling

In total, blood samples were collected from 164 herds and each farmer or livestock keeper were interviewed. In the Momba district, 132 herds were sampled and in Tunduma district, 32 herds were sampled. Within both districts the flock size varied between 1-200 sheep and/or goats.

In total 491 serum samples were collected, out of these 484 samples (98.6%) were from goats and 7 from sheep (1.4%) (Table 1). The total number of females was 405 and males 86.

Table 1. *Total numbers of animals presented in species and gender*

Animals	**Total in % (number)**
Goats	98.6 (484)
Females	81.5 (400)
Males	17.1 (84)
Sheep	1.4 (7)
Females	1.0 (5)
Males	0.4 (2)
Total	100 (491)
Females	82.5 (405)
Males	17.5 (86)

Of the total proportion of females, female goats accounted for 98.8% (400/405). The male goats accounted for 97.7% (84/86) of the total proportion of males. The female sheep accounted for 71.4% (5/7) of the total proportion of sheep, and the males for 28.6% (2/7).

All farmers reported that they owned goats and a minority reported that they owned sheep. Most farms had an average herd size between 5-15 goats (54.9% or 90/164) (Table 2). It was 4.3% (7/164) farmers that kept sheep and their average herd size were more than 15 sheep 71.4% (5/7).

Table 2. *Herd size for each species*

Species	**Herd size**	**Number (%)**
Goats	0-5	34 (20.7)
	6-15	90 (54.9)
	>15	40 (24.4)
Sheep	0-5	158 (96.4)
	6-15	1 (0.6)
	>15	5 (3)

Antibody detection

For RVFV, 3.3% (16/491) (confidence interval 95% gives 2.02-5.23%) out of the total number of animals were seropositive in the RVFV competitive ELISA on an individual level. None of the sheep was seropositive for RVFV. Out of 484 goat samples, 3.3% (16/484) was seropositive for RVFV on an individual level. The seroprevalence for female goats was 3.5% (14/400), and for male goats 2.4% (2/84), no statistic significant difference between genders (p-value 0.60). Females accounted for the larger proportion of all seropositive animals 87.5% (14/16). Totally 31.7% (13/41) of the villages had seropositive animals, the seroprevalence within the villages ranged from 0-25%. In 0.2% (1/491) of the samples the result was doubtful and in this study interpreted as negative.

Of 491 animals 75% (368/491) was older than 1 year and 25.1% (123/491) younger than 1 year (Table 3). The Momba district was 60% (296/491) older than 1 year and 20.1% (100/491) younger than 1 year. In Tunduma district it was 14.7% (72/491) animals older than 1 year and 4.7% (23/491) younger than 1 year.

Table 3. *Total number of animals and the seroprevalence for RVFV and CaPV divided by specie, gender and age*

Species	Total number (%)	RVFV seropositive (%)	CaPV seropositive (%)
Goats	484 (98.6)	16 (3.3)	1 (0.2)
Females	400 (81.5)	14 (3.5)	1 (0.3)
< 1 year	86 (17.5)	1 (1.2)	0 (0)
> 1 year	314 (64.0)	13 (4.1)	1 (0.3)
Males	84 (17.1)	2 (2.4)	0 (0)
< 1 year	37 (7.5)	0 (0)	0 (0)
> 1 year	47 (9.6)	2 (4.3)	0 (0)
Sheep	7 (1.4)	0 (0)	0 (0)
Females	5 (1.0)	0 (0)	0 (0)
< 1 year	0 (0)	0 (0)	0 (0)
> 1 year	5 (1.0)	0 (0)	0 (0)
Males	2 (0.4)	0 (0)	0 (0)
< 1 year	0 (0)	0 (0)	0 (0)
> 1 year	2 (0.4)	0 (0)	0 (0)
Total	491 (100)	16 (3.3)	1 (0.2)
Females	405 (82.5)	14 (3.5)	1 (0.2)
< 1 year	86 (17.5)	1 (1.2)	0 (0)
> 1 year	319 (65.0)	13 (4.1)	1 (0.3)
Males	86 (17.5)	2 (2.3)	0 (0)
< 1 year	37 (7.5)	0 (0)	0 (0)
> 1 year	49 (10.0)	2 (4.1)	0 (0)

Out of all seropositive animals, 93.8% (15/16) were more than one year old. Distribution between the genders is seen in Table 3.

For CaPV 0.2% (1/491) out of the total number of animals were seropositive on the double antigen ELISA. The seropositive animal was a female goat more than 1 year old in the Momba district. The goat had had signs of coughing, difficult breathing and diarrhea during the past 12 months. Positive predictive value (PPV) for a seroprevalence of 0.2% of 50% with the CaPV tests specificity of 99.7%. The negative predictive value (NPV) becomes 99.8%.

Table 4. *Individual prevalences for RVFV, CaPV and their confidence intervals (CI in the sampled districts)*

District	**Number of animals**	**Prevalence of RVFV %** (Seropositive/total number)	**CI (95%)**	**Prevalence for CaPV %** (Seropositive/total number)	**CI (95%)**
Momba	369	4.1 (15/369)	2.48-6.6%	0.3 (1/369)	0.05-1.52%
Tunduma	95	1.1 (1/95)	0.1-5.72%	0 (0/95)	0-3.89%
Total	491	3.3 (16/491)	2.02-5.23%	0.2 (1/491)	0.03-1.14%

Table 4 shows the individual prevalences for RVFV and CaPV in the two districts separately, as well as the prevalences in both districts together.

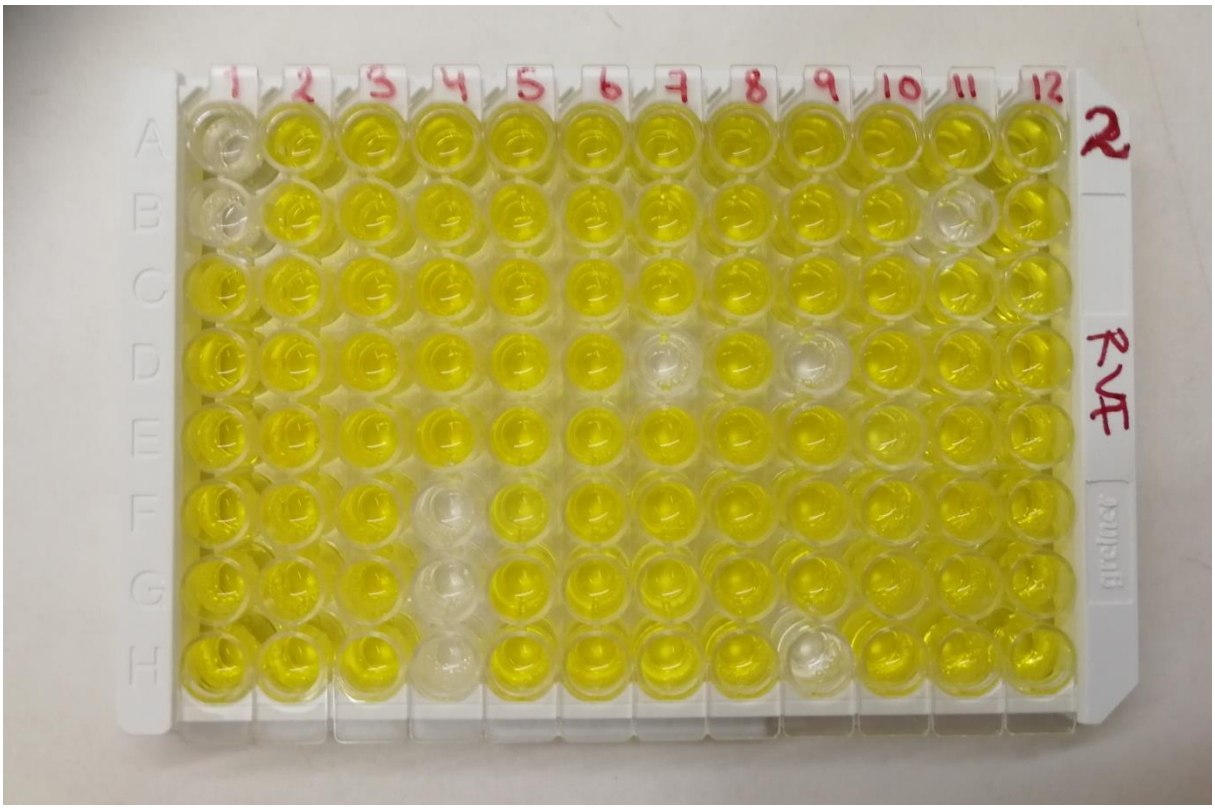

Fig 3. *A photo of an ELISA plate with 6 positive wells for RVFV* ID Screen Rift Valley Fever Competition Multi Species.

Questionnaires

Management routines

All farmers (164/164; 100%) reported that they were using communal grazing as the main grazing system for their goats and sheep. A small number of farmers also utilized herding (6/164; 3.7%) and tethering (2/164; 1.2%) in addition to communal grazing. All farmers of the sampled herds reported that their sheep and goats had daily contact with other goats and sheep and the majority (160/164; 97.6%) also reported daily contact with cattle. In contrast, almost all farmers (159/164; 97%) reported that their goats and sheep had no contact with wild ruminants.

Of the two farmers that reported that their animals were in daily contact with wild ruminants (2/164; 1.2%), one had a RVF seropositive animal. This animal was a female goat older than 1 year.

Disease managment

A majority of the farmers (157/164; 95.7%) reported that they did not vaccinate their sheep and goats. Out of those that vaccinated their animals (7/164; 4.3%) all of them vaccinated their animals against contagious caprine pleuropneumonia (CCPP).

All farmers reported that they did not keep their sick animals separated from the rest of the herd.

Trade

All farmers answered that they never had bought animals from other countries. After acquiring new sheep and goats 96.3% (158/164) immediately let them mix with the rest of the herd and only six farmers (3.7%) reported that they did not let them mix immediately. All farmers with RVFV seropositive animals let their animals mix immediately after acquiring new animals.

Table 5. *Where the farmers bought their sheep and goats from*

Location	**% (number)**
From other farmers in the same village	86.0 (141)
From farmers in other villages in the same district	42.7 (70)
From farmers in other district	3.0 (5)
At markets	18.8 (30)
From traders	1.8 (3)
Other ways	3.7 (6)

On the question of where farmers bought their sheep and/or goats from, they could answer several different options (Table 5). A majority of the farmers reported that they bought their sheep and goats from other farmers in their village. Of all the farmers, 18.3% (30/164) reported that they bought their animals from markets, and on a village level, 51.2% (21/41) of the villages had bought animals from markets. Farmers who bought their animals from markets or had neighbors who did, were more likely to have at least one RVFV seropositive animals (60.0% vs. 40%, p-value<0.000012). In the villages that bought their animals from markets, 38.1% (8/21) had at least one RVFV seropositive animals.

The farmer with the sole seropositive animal for CaPV had only bought new animals from other farmer in the same village, but the other farmers in that village reported that they had bought animals from markets.

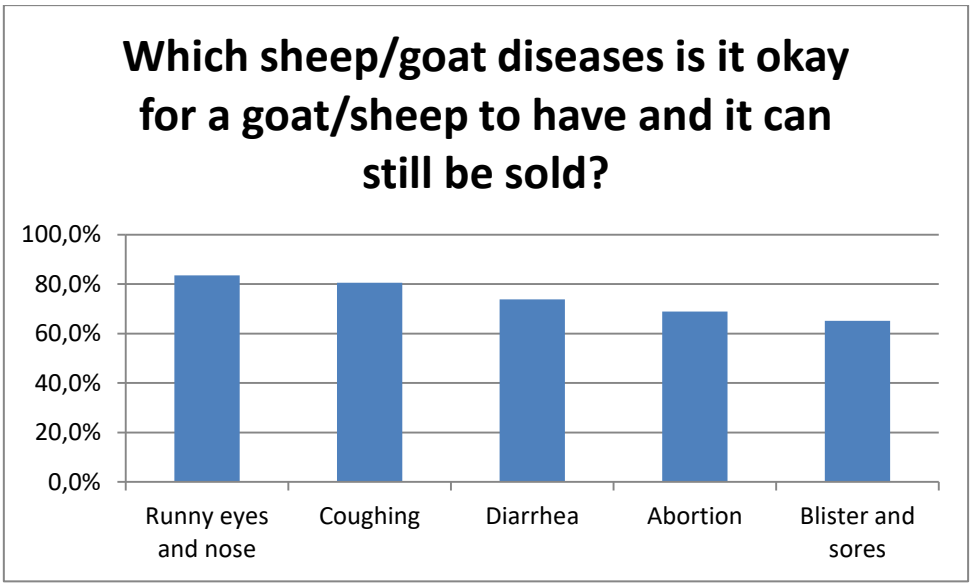

Fig 5. *The distribution of clinical signs that the farmers think was acceptable for the animals to have and still be sold.*

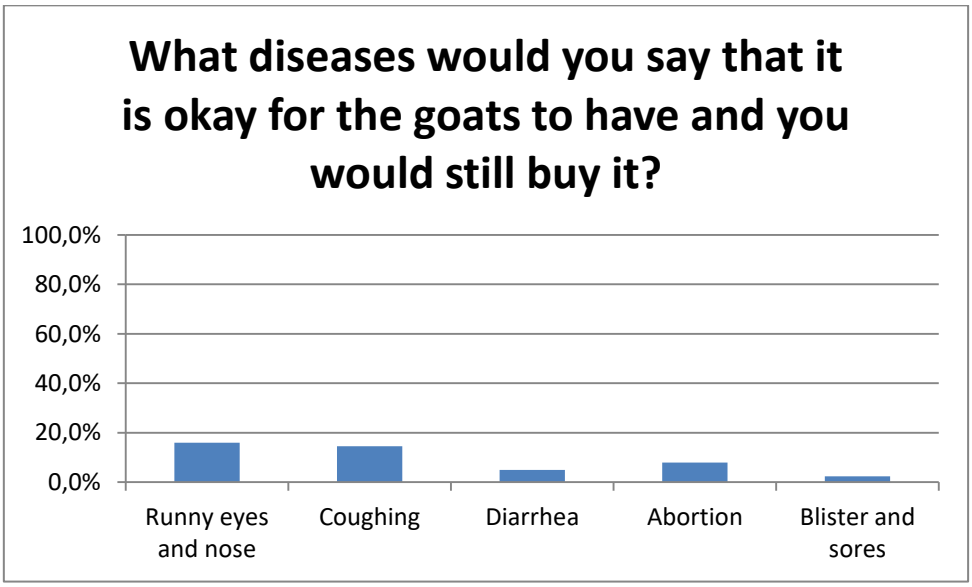

Fig 6. *The distribution of clinical signs that the farmers think it was acceptable for the animals to have and they would still buy it.*

Most farmers (458/491; 93.3%; Fig 5) reported that they think it is acceptable to sell animals with signs of ocular and nasal discharge and cough. About 15% of the farmers (Fig 6) reported that signs of ocular and nasal discharge and coughing in sheep/goats were okay for the animals to have and the farmer would still buy it.

Animal health

The most common clinical signs that farmers reported that they had observed in the last 12 months were diarrhea, coughing and ocular and nasal discharge (Table 6). The observed abortion rate was 36% (59/164) in the herds and out of the farmers with abortion during the last 12 months 13.6% (8/59) had RVFV seropositive animals. Of the farmers with seropositive

animals, 53.3% (8/15) had abortions in the last 12 months and out of those 72.7% (8/11) farmers had have abortion during the last 12 months. On a village level 42.3% (11/26) had seropositive animals during the last 12 months. There was no statistic significant difference between villages that had observed abortion and those who did not have observed any abortions.

Table 6. *The most common clinical signs that the farmer had observed in the last 12 months*

Signs of diseases	**Total number (%)**
Diarrhea	133 (81.1)
Coughing	120 (73.2)
Abortion	59 (36.0)
Dying kids/lamb	57 (34.8)
Sudden death	66 (40.2)
Blisters and sores	25 (15.2)
Runny eyes and nose	120 (73.2)

Public health

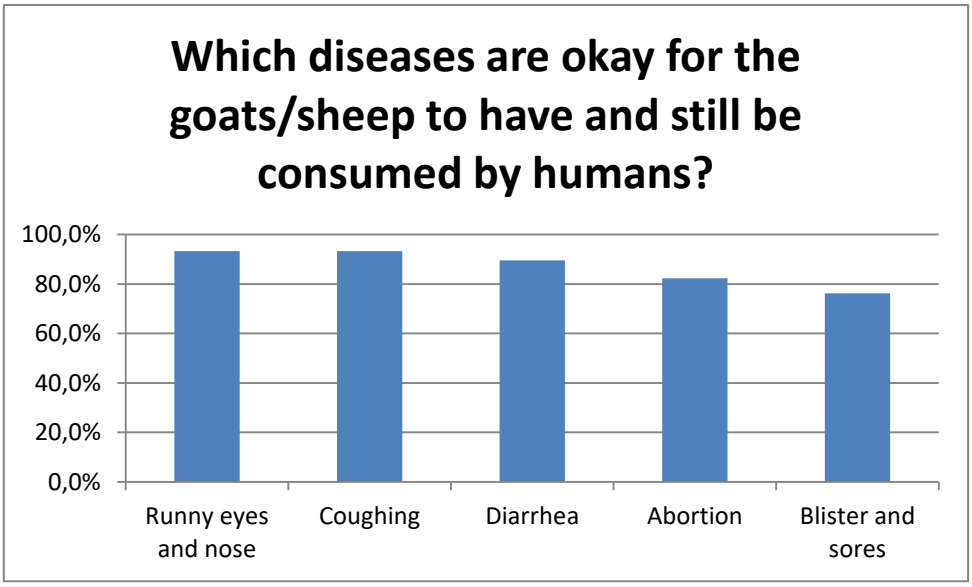

Fig 7. *The results of the question on which diseases those are ok for animals to have and still be consumed by the farmers.*

A large proportion of the farmers reported that they think it is okay to consume animals with a variety of clinical signs. Out of the signs (Fig 7) ocular and nasal discharge (153/164; 93.3%) was the most common that the farmers reported was ok for the animals to have and still be consumed. Abortion was one of the signs less frequently reported (135/164; 82.3%) as okay for the animals to have and still be consumed. Of the farmers with seropositive animals, 80% (12/15) of them reported that they did consume animals that had aborted. There was no statistic significant difference between the two groups.

Farmer details

Table 7. *Gender and age of farmer and/or caregiver of the animal and the seroprevalence*

Age	% (number)	Seropositive % (number)	Years in school	% (number)	Seropositive % (number)
Women	100 (41)	4.9 (2)	**Women**	100 (41)	4.9 (2)
<30	22.0 (9)	0 (0)	0 years	29.3 (12)	0 (0)
31-50	63.4 (26)	7.7 (2)	1-7 years	63.4 (26)	3.8 (1)
> 51	14.6 (6)	0 (0)	> 7 years	7.3 (3)	33.3 (1)
Men	100 (122)	10.7 (13)	**Men**	100 (122)	10.7 (13)
<30	14.8 (18)	16.7 (3)	0 years	21.3 (26)	19.2 (5)
31-50	63.1 (77)	10.4 (8)	1-7 years	67.2 (82)	8.5 (7)
> 51	22.1 (27)	7.4 (2)	> 7 years	11.5 (14)	7.1 (1)

Among all farmers, 23.2% (38/164) reported that they had not gone to school. Out of the farmers, 9.1% (15/164) of them had RVFV seropositive animals and of all farmers with seropositive animals, 33.3% (5/15) was uneducated.

Table 8. *Univariable analysis for risk factors associated with seropositivity for RVF at an individual animal level*

Questions	**Answers**	**RVF Seropositive**	**RVF Seronegative**	**P value < 0.05**
Have you observed any symptoms in your sheep/goat in the last 12 months?	Yes (149)	15	431	0.681174
	No (15)	1	44	Not significant
Where do you buy sheep and/or goats from?	Within the district (136)	14	393	0.618746
	Outside the district (28)	2	82	Not significant
When was the last time you bought sheep and/or goats?	Within the last 6 months (29)	2	85	0.578305
	More than 6 months ago (135)	14	390	Not significant
After acquiring new sheep and goats, do you let them mix with your original heard immediately?	Yes (158)	16	457	0.739461
	No (6)	0	18	Not significant

DISCUSSION

In this master thesis, the aim was to investigate the seroprevalence of RVFV and CaPV among sheep and goats in the Momba and Tunduma districts, close to the border of Zambia. The individual seroprevalence for RVFV in sheep and goats in the two districts were 3.3% (16/491), no statistic difference was observed between the two districts. Only 1.4% of the animals included in this study were sheep and none was seropositive, but the population included was too small and no conclusion could be drawn. In Momba district the prevalence on a village level was 33.3% and for Tunduma 25%. This seroprevalence indicates that sheep and goats sometime during their lifetime have encountered RVFV due to either natural infection or through vaccination, the latter one is in this study considered unlikely. In this study, only goats were seropositive for RVFV, but a majority of the animals (98.6%) included in this study was also goats. Previous studies have identified sheep to have a higher seroprevalence of RVFV than goats (Blomström *et al*., 2016; Jeanmaire *et al*., 2011; Rostal *et al*., 2010). In Tanzania, a slightly higher number of goats than sheep have been seropositive in some district (Wensman *et al*., 2015). In other studies, no significant differences between the two species been were observed (Sumaye *et al*., 2013).

In this study, 3.8% of the females were seropositive compared to 1.2% of the males, but no significant difference between genders was observed. Out of all seropositive animals, all but one were more than one year of age, 14 being females and one male. The only seropositive animal under 1 year was a female. This could indicate presence of the virus with ongoing circulation of RVFV in the districts. Previously conducted studies detected young animals to be seropositive showing continuous circulation of RVFV in the northern and central part of Tanzania during an inter-epidemic period (Wensman *et al*., 2015). Sindato *et al*. (2014) concluded in their review that RVF outbreaks were mainly reported during periods of prolonged heavy rainfall. Sindato *et al*. (2010) suggest that when RVFV has been introduced to a new geographical area, it becomes endemic and favorable conditions in the environment allow a reactivation in a large scale. It is possible that RVFV is circulating in the Momba district where the animal under 1 year was found seropositive.

The age of the animals was estimated mainly based on the farmers' information. Some owners knew exactly how old every individual was, while some farmers were not sure about the age and gave an approximated age. The animals included in this study would have to be over 4 months old, in some cases when the farmer was unsure about the age and the size of the animal was tiny, it was rejected too avoid sampling animals that were too young. Some animals were estimated by the farmer to be around one year. This could influence the interpretation of the seroprevalence between the age group, when results are presented under or above one year old.

A supposed risk factor for transmission of RVF is contact with wildlife (Wensman *et al*., 2015). In this study, most farmers reported that there was no direct contact between sheep and goats and wild ruminants. Almost all farmers reported that their sheep and goats were in daily contact with cattle, and these animals graze further away from the livestock keepers and can therefore

come in contact with wild ruminants. This could be a risk factor of transmission of RVFV between domestic ruminants and wild ruminants.

For RVF, abortion storms are the clinical manifestation that is most significant (OIE, 2014). In this study, the observed abortion rate within the herds was 36%, and out of this 53.3% of the farmers with seropositive animals had abortions in the last 12 months. No significant difference was detected for farmers with seropositive animals who also had reported that they had abortions during the last 12 months.

Rift Valley fever virus is a zoonotic disease (Reed *et al*., 2012). Transmission of the virus can occur through arthropods vectors and from organs and body fluids of infected animals (OIE, 2009). Occupational groups such as farmers, slaughterhouse workers and veterinarians are at higher risk of infection (WHO, 2018), with symptoms such as acute fever, headache, and pain in muscles and joints (Ikegami, 2013). These symptoms often decline after 4-7 days if no complication arises, in some humans, complications such as bleedings, liver failure and encephalitis occur. In previous studies in the Mbeya regions, that include Momba and Tunduma district, a seroprevalence at 5.2% has been observed in humans (Heinrich *et al*., 2012). Abortions were less frequently reported (82.3%) acceptable for the animals to have and still be consumed reported. However, 80% of the farmers with seropositive animals did report that they did consume animals that had aborted. This is a possible risk factor associated with transmission of disease since contact with organs and body fluids of infected animals, and the placenta can contain high virus titers (Pepin *et al*., 2010; OIE, 2009) This indicates an unawareness of the risk or that they have to consume the sick animals out of poverty, and it can therefore lead to recycling of RVFV between humans and animals.

Capripoxviruses are endemic in Africa north of the equator (OIE), and in Ethiopia World Organization of Animal Health Information Database has documented occurrences of SGP since 1996. A previous study in Congo has shown a seroprevalence of 52.7% of CaPV (Bwihangane *et al*., 2017). In this case, it was an outbreak with both CaPV and peste des petits ruminants. In a study presented in 2017 from Ethiopia, a seroprevalence of 17% in sheep and 14% in goats was reported (Fentie *et al*., 2017). No reports of CaPV have been published in Tanzania until 2018, when CaPV was detected during a massive outbreak of respiratory disease in 2016 (Kgotlele *et al*., 2018). This outbreak affected sheep and goats in the Ngorongoro district in the north of Tanzania. It was confirmed that in this outbreak, occurrence of co-infection with pathogens associated with respiratory distress such as PPR, where present. Within this study, there was only 0.2% seropositive animals for CaPV, a single female goat over 1 year old. Within the last 12 months the farmer reported that she had signs of coughing, difficult breathing and diarrhea. The farmer utilized communal grazing and the livestock had daily contact with other sheep and goats and also cattle, but never contact with wild ruminants. The farmer reported that animals were bought once a year from other farmers in the village and villages in the district, but also at markets. A risk factor seen in other studies is movement of livestock (Mangana *et al*., 2008), and it could possibly be an explanation for the presence of a seropositive animal in this study.

It is important to take in consideration that this positive sample could in fact be a false positive, meaning that this study could not identify presence of antibodies to CaPV in sheep and goats. If the observed seroprevalence of 0.2% is considered to be the true prevalence of CaPV, the positive predictive value (PPV) would be 50% with the CaPV tests specificity of 99.7%. The high specificity together with the low prevalence will give a large number of animal that are false positive. The negative predictive value (NPV) would be 99.8%, meaning that it is a high possibility of an individual with a seropositive value not to be infected with the virus.

Further knowledge of CaPV is needed, the OIE addresses more information about CaPV and the need for better understand, not only epidemiology, transmission and immunity but also focus on development of effective prophylactic tools (Tuppurainen *et al*., 2015).

For farmers in rural communities both of these diseases can have significant negative socio-economical impact due to the loss of production and animals. The gender-equality between men and women may also be affected since women often are the main caretaker of the livestock. Future studies are acquired to understand more about these diseases and their epidemiological present in Tanzania.

In this study it is important to note that findings may have been affected by sampling technique, season of sampling and sample size. Other thing that may affect the study is misunderstanding of language, because Tanzania consists of many different ethnical groups and they speak more than 100 languages, lead to misconception of the questions. When answering the questions, they could also have been influenced by other farmers or people living in the village, this because often a large number of people was gathered when blood collection was performed.

CONCLUSIONS

This master thesis suggests that RVFV is serologically (3.3%) present in goats in the southwestern part of Tanzania close to the border of Zambia. The laboratory results suggest that the disease is present and continuously circulating in the population since both young and old animals were seropositive. The seroprevalence most likely indicates natural infection of the animals.

Only one CaPV seropositive animal was identified and no assumptions could be made about the presence of the disease in Tanzania.

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POPULÄRVETENSKAPLIG SAMMANFATTNING

I detta examensarbete undersöktes förekomsten av sjukdomarna Rift Valley feber (RVF) och får- och getkoppor (SGP) hos får och getter. Insamlingen av material till examensarbetet genomfördes i sydvästra Tanzania, nära gränsen till Zambia.

Tanzania är ett land där fattigdomen fortfarande är hög och många hushåll är beroende av jordbruk för att försörja sin familj. Får och getter utgör en viktig del av jordbruket då de är billiga att köpa in för bönder och också lätt kan säljas eller bytas. Djuren blir därmed en slags levande bank och skulle en oförutsedd utgift uppstå så kan ett djur säljas för att få kontanter. Detta innebär att för bönderna är det viktigt att dessa djur håller sig friska och inte förloras i någon sjukdom.

De två sjukdomarna RVF och SGP är virussjukdomar som framförallt drabbar får och getter. Djuren kan smittas på flera olika sätt. Det finns vissa likheter mellan dem men också skillnader. För RVF smittas djuren framförallt genom att myggor som bär på viruset biter och suger blod från djuren och då kan viruset föras över till fårets/getens blod och djuret blir infekterat. Det finns många olika arter av myggor som kan bära på viruset och en del av dessa lägger ägg som kan överleva flera år i miljön. Detta gör att smittan kan leva kvar länge i olika områden. Får- och getkoppor smittar genom direktkontakt mellan infekterade djur och friska djur. De sjuka djuren sprider virus genom kroppsvätskor som kommer från nos och/eller munhåla som det friska djuret kommer i kontakt med. Virus som orsakar SGP är tåliga och kan överleva länge i miljön. Det gör det möjligt att friska djur som kommer i kontakt med miljö som är kontaminerat av virus kan bli sjuka. Rift Valley feber virus (RVFV) kan också föras över genom att friska djur kommer i kontakt med kroppsvätskor eller aborterade foster som innehåller höga nivåer med virus.

Vid båda sjukdomarna påverkas djurens allmäntillstånd negativt och kan i vissa fall ha en dödlig utgång. För Rift Valley feber är det främst de unga djuren som drabbas och dödligheten bland dem är hög, vilket betyder att alla unga djur kan förloras om sjukdomen drabbar dem. Djur som är dräktiga, oavsett när i dräktigheten, kan abortera sina foster. Detta brukar ses i form av så kallade abortstormar och är det sjukdomstecken som är specifikt för denna sjukdom. Får- och getkoppor kan också ha ett akut sjukdomsförlopp med feber, aptitlöshet, ökad salivering och att det rinner från nos och ögon på de sjuka djuren. Efter ett par dagar ses röda utslag i huden, koppor, och även på slemhinnor som till exempel i munnen. Det är lättast att se kopporna där ullen/pälsen är som tunnast och även i munhålan. Kopporna börjar som små röda prickar och blir sedan blåsor som vätskar. Därefter bildas krustor när läkning sker. De blåsor som bildas i slemhinnan i munnen eller i andra delar av slemhinnor inne i kroppen kan ge allvarliga komplikationer. Det beror på att bakterier då kan få fäste och därmed ge infektioner och försvåra läkningen. Av denna anledning ses den höga dödligheten bland unga djur.

Det finns risk att människor insjuknar i RVF då viruset kan överföras från djur till människor, det brukar kallas för att sjukdomen är en zoonos. Hos människor ses ofta RVF hos personer som är i nära kontakt med smittade djur och deras organ och kroppsvätskor, men människor, precis som djuren, smittas även genom myggor. Sjukdomen har vanligen ett kort förlopp med

symtom som plötslig feber, smärta i leder och armar och ben. I vissa fall förekommer det att komplikationer tillstöter såsom hjärnhinneinflammation och leversjukdom och i vissa fall kan det leda till döden. För SGP är det en väldigt liten risk att människor drabbas. Ett fåtal fall har beskrivits och då har personerna som hanterat djuren fått koppor på händerna.

I detta examensarbete genomfördes en fältresa till två distrikt i Tanzania, Momba och Tunduma, som ligger nära gränsen till Zambia. Totalt provtogs 41 byar i de båda distrikten. I Momba provtogs 33 byar och i Tunduma provtogs 8 byar. I varje by besöktes 4 bönder och hos varje bonde provtogs 3 djur. Totalt för en by blev det alltså 12 djur som provtogs och med alla byar blev det totalt 491 blodprover, ett blodprov saknas då det försvann under fältresan. De bönder som ingick i studien valdes ut genom en urvalsmetod som fungerar likt en snöboll. I denna metod går man fram till en bonde i byn och tar blodprover från dennes djur, i sin tur får bonden peka ut en annan bonde med får och getter som är lämpliga att ingå i studien. För studien fanns tre kriterier, dessa användes i varje by för att kunna besöka bönder med olika flockstorlekar:

- Bonde med mindre än 5 får och/eller getter
- Bonde med 5-15 får och/eller getter
- Bonde med fler än 15 får och/eller getter

Blodprovstagningen gick till på följande sätt: en stor ven på sidan av halsen synliggjordes genom att ett bestämt tryck placerades vid halsens nedre del. När venen blev synlig stacks en vass kanyl genom huden och in i venen. På kanylen fanns en så kallad vacutainer påkopplad, som gör det möjligt att koppla på ett provtagningsrör med ett undertryck utan att blod rinner direkt ut ur kanylen direkt på marken innan provtagningsröret kopplas på. De provtagningsrör som användes innehåller inga ämnen, vilket gör att blodet koagulerar och kvar finns vätskan (serumet) som bland annat innehåller antikroppar. Denna vätska togs ut ur varje blodprovsrör när det hade koagulerat klart och placerades i egna mindre rör. Dessa förvarades i en frys tills att alla prover tagits ute i de båda distrikten. Från varje djur som provtogs samlades data in om hur gammalt djuret var, vilken art (får eller get), vilket kön, om det varit sjukt tidigare (inom 12 månader) och om den hade några tecken på sjukdom när blodprovet togs. För att kunna koppla ihop varje djur med rätt blodprov så märktes både röret och svaret med samma siffror.

När alla prover samlats ihop påbörjades analyseringen av dem i Morogoro. Analyseringen av serumet gjordes i ett laboratorium med hjälp av ELISA-kit, dessa kit används för att upptäcka antikroppar. Dessa antikroppar bildas när kroppens immunförsvar kommer i kontakt med ett virus. Antikropparna är unika och kommer bara binda till just det viruset som de bildats mot. Genom att identifiera antikroppar så kan det visa ifall djuret varit i kontakt med viruset tidigare. Eftersom antikropparna har ett unikt utseende beroende på vilket virus de har utvecklats mot kommer antikroppen att ha ett eget utseende som dessa specifika ELISA-kit kan upptäcka.

Antalet djur var totalt 491 varav 484 getter och 7 får. Av dessa var 405 hondjur (getter/tackor) och 86 handjur (bockar/baggar). Andelen djur där antikroppar hittades, var 3.3% för RVF. Inga får var positiva och av getterna så var 93.8% getter och resterande bockar. Av de positiva djuren var ett under ett år vilket kan tala för att viruset finns i omgivningen och smittar unga djur. Detta

stämmer med vad man hittat i tidigare studier där man undersökt förekomsten av RVF i Tanzania.

I denna studie sågs en skillnad mellan bönder som hade positiva djur för RVFV och de som inte hade positiva djur avseende om de köpte sina djur från marknader eller om det fanns grannar i samma by som gjorde det. Det betyder att det var en risk om bonden själv köpte djur från marknader eller att bönder i samma by gjorde det. De köpta djuren skulle kunna ha haft sjukdomen. Samtliga bönder uppgav att de inte höll nya djur separerade från resten av flocken utan släppte ihop dem direkt. Detta ökar risken för smittspridning.

I de båda distrikten så uppgav många bönder att de släppte ut sina djur under dagen och lät dem beta fritt. Djuren kom då ofta i kontakt med andra bönders får och getter, men också med nötkreatur. Det var sällan som böndernas får och getter hade någon kontakt med vilda idisslare. När djuren blev sjuka så var det ingen bonde som separerade det sjuka djuret från resten av flocken utan alla djur hölls tillsammans.

Det var ett djur som var positivt för får- och getkoppor. Det positiva djuret var en get som var över ett år gammal. Bonden hade själv inte köpt några djur från djurmarknaden men de resterande tre bönder i byn som intervjuades uppgav att de hade köpt djur från marknaden. Får- och getkoppor har visat sig kunna spridas genom att djur flyttas och därmed skulle en djurmarknad vara en risk för att sjukdomen sprids mellan områden. Eftersom det enbart var ett positivt djur så kan det vara ett falskt positivt resultat (att provet visar ett felaktigt värde).

Denna studie bidrar med ökad kunskap om förekomsten av såväl Rift Valley feber och får- och getkoppor i Tanzanias sydvästra del, på gränsen mot Zambia. Den låga förekomsten talar för att Rift Valley feber förekommer i området och att det finns positiva djur under ett år talar för att viruset regelbundet cirkulerar.

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APPENDIX 1

Management routines:

- What grazing system are you utilizing?
- How often are your sheep and/or goats in contact with sheep and goats from other herds?
- How often are your sheep and/or goats in contact with cattle from other herds?
- How often are your sheep and/or goats in contact with wild ruminants?

Medicine:

- Do you vaccinate your sheep and/or goats?
- When one or few of your sheep and goats are sick, do you keep it/them separated from the rest of the herd?

Trade:

- When was the last time you bought/bartered or in any other way acquired sheep and/or goats to include in your herd?
- Where do you buy sheep and/or goats from?
- Have you ever bought sheep and/or goats from other countries?
- After acquiring new sheep and goats, do you let them mix with your original herd immediately?
- When was the last time you sold sheep and/or goats?
- Which sheep/goats diseases is it OK for a goat/sheep to have and it can still be sold?
- What diseases would you say that it is OK for the goat/sheep to have and you would still buy it?

Animal health:

- What signs of diseases did you observe in your sheep and/or goats, in the last 12 months?

Public health:

- Which diseases are OK for the goat/sheep to have and still be consumed by humans?

Details of goats/sheep owned:

- Herd size in goats and sheep (adult, males, females and kids/lambs)

Farmers' details:

- Gender
- Age
- How many years have you been in school?