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Faculty of Veterinary Medicine and Animal Science

Zoonotic diseases in Zambian goat herds

Prevalence and risk factors



Pernilla Karlsson Bergkvist

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Pernilla Karlsson Bergkvist

Supervisor: Jonas Johansson Wensman, Department of Clinical Sciences.

Assistant Supervisors: Sara Lysholm, Department of Clinical Sciences and Musso Munyeme, University of Zambia.

Examiner: Madeleine Tråvén, Department of Clinical Sciences.

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SUMMARY

Small ruminants play an important role in low-income countries due to their ability to bring food and income to their owners. Loss of these animals due to disease can therefore affect a family's ability to support their livelihood and the children's opportunities to go to school so that they can improve their future. The aim of this study was to investigate the prevalence of three zoonotic diseases among goats that can have an impact on both animal health and production and human health: brucellosis, Crimean-Congo haemorrhagic fever (CCHF) and Rift Valley fever (RVF). Another aim was to find associations between the seroprevalence of the disease and management routines to see if there were any specific risk factors to contracting the diseases in the herds.

Serum samples were collected from goats in three districts in the Central and Southern provinces of Zambia, more specifically Monze and Mazabuka in the Southern province, and Chibombo district in Central province. In each district, ten villages were randomly selected and in each village four households with at least four goats were visited for sampling. The farmers in each household were interviewed using a questionnaire with questions regarding management routines and details about the animals.

After sampling the serum was analysed for antibodies to the selected diseases using commercially available specific ELISA kits. To find associations between the seroprevalence and management routines, Fisher's exact test was used.

No individuals were found to have RVF antibodies. The prevalence of CCHF was found to be 5.2% and seroprevalence of brucellosis was 2.7%. Association was found between being CCHF seropositive and having contact with other cattle herds equal to or more often than every six months. No other associations could be found between seropositive animals and management routines.

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ABBREVIATIONS

CCHF – Crimean-Congo haemorrhagic fever CCHFV – Crimean-Congo haemorrhagic fever virus RVF – Rift Valley fever RVFV – Rift Valley fever virus WHO – World Health Organisation

INTRODUCTION

Small ruminants play an important role in impoverished or low-income societies in rural and peri-urban areas in developing countries. Because they are often cheaper to acquire than cattle, they are an important source of food and money for their owners. Loss of these animals due to disease can cause a noticeable effect on the farmer's livelihood (FAO, 2013). Implementing animal health programmes in poor countries has been proven to help farmers who might otherwise be affected by trade-bans or reduced payment because of sick animals (Nin Pratt *et al.*, 2005).

Zambia is a landlocked country in southern Africa. It has eight neighbouring countries. The country has experienced recurrent droughts that have led to increased food insecurity. Half of the children under the age of five suffer from malnutrition. Most of the population is dependent on small-scale agriculture for their livelihood. Zambia experienced a strong economic growth during the 2000's but there are still many people living in a state of poverty (Globalis, 2013).

The aim of this study was to increase the understanding of how common the zoonotic diseases Rift valley fever, brucellosis and Crimean-Congo haemorrhagic fever are among goats in Zambia. These three diseases can spread between humans and animals making them important in both an economical and human health aspect. To investigate the prevalence of the diseases, serum samples were collected from 480 goats dispersed in three districts in central and southern Zambia. Sera were then analysed with ELISA to search for antibodies to these infectious agents, giving an indication of how many of the animals that had been in contact with these pathogens.

Another aim was to identify risk factors for contracting and spreading these diseases among the goats. This was done by comparing answers from the farmers in a questionnaire with the ELISA-results.

LITERATURE REVIEW

Brucellosis

Brucellosis is a bacterial infection caused by the bacteria-family *Brucella*. The different bacteria within this family tend to prefer a specific species of animal but are also very infectious zoonoses (OIE Brucellosis, 2019). The most common type of *Brucella* in goats and sheep is *Brucella melitensis*, but they can occasionally be infected by *B. abortus*.

The disease is widespread, but areas at higher risk of brucellosis are the countries of the Mediterranean Sea Basin, South and Central America, Asia, Africa, the Caribbean and Near East, most likely due to less developed public and animal health services. Infected animals, both domestic and wild, are the main source of infection for humans. Therefore, farmers, butchers and veterinary personnel are considered more at risk for infection (Galinska and Zagórski, 2013). In goats, the diagnosis can be confirmed through bacterial examination of milk or aborted foetuses. A serum agglutination test can also be performed (Merck & Co, 2019).

The bacterial infection causes abortions and it spreads mainly from the amniotic fluid and vaginal secretions during abortion or birth. The bacteria also have the potential to colonize the udder in the animal and spread to offspring and humans through unpasteurized milk. The disease causes great losses to farmers because of reduction in fertility in the herd and the birth of weak offspring with a higher mortality rate (OIE Brucellosis, 2019). There is an association between higher prevalence of brucellosis and higher losses in farming productivity. This loss in productivity depends on higher rates of abortions, stillbirth and longer intervals between pregnancy in the females. In cases where the animals are kept for milk the milk, yields will lessen due to the reduction in fertility (Nitrandekura *et al.*, 2018).

A study in Iraq investigated risk factors for brucellosis among sheep and goats (Alhamada *et al.*, 2017). The authors found that animals in mixed sheep and goat herds often had a higher prevalence of antibodies to *Brucella* spp., and suggested this to be due to mixed herds often consisting of more animals. Additionally, they found that older animals more often were seropositive. This was attributed to the fact that older animals have been reproductively active during a longer time compared to younger animals and therefore exposed to the risk of contracting the disease during a longer period (Alhamada *et al.*, 2017). Another study also found that larger herds and higher parity number increased the risk of being seropositive for *Brucella* spp. and that goats were more susceptible to the disease than sheep (Tegegn *et al.*, 2016). A study performed on Zambian cattle could likewise determine that a larger herd size increased the risk of being seropositive for *Brucella* spp. This study also revealed that exposure to wild animals increased the risk of being seropositive (Muma *et al.*, 2007). In Zambia, it was shown that moving cattle between different grazing areas is a bigger risk for seroprevalence than other grazing systems (Muma *et al.*, 2006).

Clinical signs, prevention and control

Animals show few signs of disease before abortion. In some cases, the bacteria can spread, and cause arthritis, while males can develop a swelling of the reproductive organs. In epidemic

areas, vaccination can be used to reduce the number of sick animals. The disease is difficult to eradicate because of wildlife being a reservoir for the bacteria (OIE Brucellosis, 2019). A study in Zambia from 2010 found that 21.6% of the Kafue lechwe, a local wild ruminant, had antibodies to *Brucella* spp. (Muma *et al.*, 2010).

The incubation period in humans is between two and four weeks. Four different species of *Brucella* can infect humans; *B. melitensis, B. abortus, B. suis* and *B. canis*, but the most common is *B. melitensis*. The infection can be of acute, subacute or chronic form (Pappas *et al.*, 2005).

The acute form of the disease in humans can present itself as undulating fever, weakness, sweating, headaches, enlarged liver and spleen, vomiting, diarrhoea, weight-loss and general aching (OIE Brucellosis, 2019; Pappas *et al.*, 2005; Galinska and Zagórski, 2013). The acute phase may end in death, recovery or may progress into a chronic or sub-acute form. The sub-acute form consists of the same symptoms but not as strongly expressed. In the chronic form there might not be any symptoms at all (Galinska and Zagórski, 2013).

Sweating with a foul odour is pathognomonic for brucellosis. Enlarged spleen, liver and lymph nodes are common. In pregnant women brucellosis often results in spontaneous abortions. Neurologic complications to the infection can occur, such as demyelisation, meningoencephalitis or brain abscesses, although, most deaths due to the disease in humans are caused by endocarditis. The treatment consists of broad-spectrum antibiotics during three to six weeks. In cases of endocarditis a valve replacement surgery is often required in the early stages to save the patient's life (Pappas *et al.*, 2005).

The broad spectrum of symptoms give rise to difficulty in making the diagnosis. Usually the bacteria can only be cultivated from a patient in the acute form, and even then, this can be difficult (Galinska and Zagórski, 2013).

Human vaccines have been developed and tested in the past, but none has been accepted as safe, probably because the entire pathogenesis of brucellosis has not yet been fully understood (Pappas *et al.*, 2005). In 2007, Zinsstag *et al.* reviewed the effect that vaccination in animals has on human health and the cost-benefit, with an example taken from Mongolia. Their conclusion was that if they could achieve 52% reduction of brucellosis transmission between animals in Mongolia, by vaccinating both cattle and small ruminants, this would reduce the human cases by approximately 51 800 annually, making it one of the most cost-effective interventions in the health sector (Zinsstag *et al.*, 2007). Although this cannot be fully translated to the Zambian community, it suggests that vaccination among animals is beneficial in reducing transmission of disease.

Crimean-Congo haemorrhagic fever virus

The Crimean-Congo haemorrhagic fever virus (CCHFV) belongs to the family *Bunyaviridae* and is spread throughout Africa, Asia, the Middle East and south-eastern Europe. The virus circulates between ticks and vertebrates, especially ticks of the genus *Hyalomma*. Animals infected with the virus very rarely show signs of disease (OIE Terrestrial manual, 2018). The

virus remains in the bloodstream of an infected animal for up to a week, allowing the virus to transfer to other ticks which helps the disease to spread (WHO, 2013).

Humans can be infected from ticks, body fluids from animals with viremia and may spread from person to person with body fluid and blood (OIE Terrestrial manual, 2018; Smego *et al.*, 2004). A recent study of an outbreak in central Uganda in 2017 showed that people with clinical symptoms of the disease had been in contact with seropositive cattle and goats prior to infection (Kizito *et al.*, 2018). Because of the disease spreading between people, the risk of disease is likewise increased among healthcare personnel (Smego *et al.*, 2004).

The pathogenesis of the disease in humans is currently not well understood. Antibody seroprevalence in animals is a good indication for virus circulating in an area and can help to focus health interventions (OIE Terrestrial manual, 2018). The diagnosis can be confirmed by using ELISA, real-time PCR or to try and isolate the virus from blood or tissue samples. Real-time PCR and virus isolation are only feasible early in the infection (CDC, 2013).

Clinical signs, prevention and control

Animals show a mild fever and have viremia for up to one week after infection (OIE Terrestrial manual, 2018). Apart from the fever, animals do not show signs of disease but act as an amplifying host of the virus (Fillâtre *et al.*, 2018).

In humans, the incubation period depends on the source of infection. From ticks, the onset of disease is usually one to three days, while infection from slaughtered animals and animal products cause symptoms in about five to six days (WHO, 2013). The onset of disease is sudden, with symptoms such as fever, myalgia, dizziness, neck pain and stiffness, headache, sore eyes and photophobia. It may cause abdominal pain and liver enlargement or agitation that over time is replaced by depression (WHO, 2013). There may be bleeding in the skin and mucosa, ranging from small petechiae to large haematomas, with mortality rates between 40 - 80% (OIE Terrestrial manual, 2018; WHO, 2013). Death typically occurs in the second week of symptoms while signs of recovery usually occur in the ninth or tenth day of disease (WHO, 2013).

Today there are no safe, efficient vaccines available against this disease, not for humans nor animals (OIE Terrestrial manual, 2018; WHO, 2013). In the 1970s, a vaccine was developed in the Soviet Union and used amongst military and agricultural workers. The vaccine later proved to have a low efficacy and demanded three boosters to give any effect (Dowall *et al.*, 2017). Another vaccine derived from mouse brains caused autoimmune effects and therefore is not considered safe to use in humans (Dowall *et al.*, 2017). It has been investigated if the antiviral drug ribavirin in combination with supportive treatment could lower the mortality and shorten time in hospital care, but evidence toward this is low and it is not certain what the adverse effects of the medication are (Johnsons *et al.*, 2018). Although, ribavirin and supportive treatment is still recommended by the World Health Organisation (WHO, 2013).

Regular use of acaricides is a good way to keep the number of ticks down, resulting in a lower risk of infection (OIE Terrestrial manual, 2018). It may prove difficult since the disease among animals usually goes unnoticed and the acaricides require regular application and good control of animals (WHO, 2013). It is also important to be careful in handling animals in situations such as slaughter, because of the exposure of blood from animals with viremia, to lower the risk of human infection (OIE Terrestrial manual, 2018).

Rift Valley fever virus

Rift Valley fever virus (RVFV) is an RNA virus of the family Bunyaviridae. The disease is vector-borne and mainly affects goats, sheep and cattle, but wild ruminants and humans are also susceptible to the virus. The virus spreads with many different species of mosquitoes, such as Aedes, Anopheles, Culex, Eretmapodites and Mansonia, among others (OIE Technical disease card, 2019). The virus can survive in the eggs of the Aedes-species (OIE Technical disease card), making this the main reservoir. In endemic areas, the disease can circulate between mosquitos and ruminants without apparent clinical signs. While in dry areas, periods of heavy rains can cause an outbreak following a mass-hatching of mosquitoes, with more severe signs in animals that might be exposed for the first time (OIE Technical disease card). These periods of outbreaks are called epizootic periods. They occur when the rainfall is extra persistent and causes areas of usually dry land to flood and puddles to persist for four to six weeks. This causes the mosquito eggs to hatch in large numbers and spread the disease in a more rapid way (FAO, 2003). These epizootic periods can last for one to three years and then the disease diminishes for some time due to the water levels lowering again together with the number of vectors. These periods of dryer weather with few signs of circulating disease are called inter-epizootic periods and can last for five to fifteen years (Davies et al., 1985). Antibodies to the virus can be detected approximately one week post-infection. These antibodies give long-lived protection in all species and some sheep and cattle have been shown to be totally resistant against re-infection (Wright *et al.*, 2019).

Humans can get infected directly from mosquitoes as well as an infected animal. Contact with nasal discharge, blood or vaginal secretion after abortion in animals, infected meat and possibly consumption of unpasteurised milk are sources of infection (OIE Technical disease card, 2019). Even the act of milking an infected animal could be a risk factor for human infection (Grossi-Soyster *et al.*, 2019). A study performed in Kenya discovered that being over the age of 15, of male gender and working with animal products, were factors that increased the risk of being RVFV infected (LaBeaud, 2015). People that were living closely to their animals and that were involved in animal slaughter and the disposal of aborted foetuses had elevated risk (LaBeaud, 2015).

The period of viremia after infection is short and therefore diagnosis through identifying the virus in blood samples can only be performed in the first five days. For this analysis, real-time PCR is the most common and reliable method. After a few days of infection, the infected host starts to produce antibodies to the virus. Initially, it is the IgM-antibodies that dominate but after approximately 20 days the IgG-antibodies are more numerous. During this phase, ELISA

or virus neutralization test are more commonly used to identify specific antibodies and confirm the disease (Lagerqvist, 2013).

Clinical signs, prevention and control

Incubation period varies from 1–6 days. Lambs and kids are considered extremely susceptible to the disease with a mortality of 70–100%. Sheep and calves have a mortality rate of 20-70% and cattle, goats, buffaloes and humans have a mortality rate under 10% (OIE Technical disease card, 2019).

Goats present clinical signs such as high fever, anorexia, weakness, depression, increased respiratory rate, mucopurulent nasal discharge, vomiting, bloody diarrhoea, and in pregnant animals, abortion (OIE Technical disease card, 2019). The abortion rate seems to vary between the documented outbreaks. Usually, the abortion rate is estimated between 40-100% but in an outbreak in Egypt in the late 1970s, goats were seemingly resistant (Lagerqvist, 2013).

Humans can have an influenza-like syndrome with fever, headache, muscular pain, photophobia and weakness. In some cases, complications can occur, such as retinopathy, blindness, meningo-encephalitis or haemorrhagic syndrome. The disease is in some cases lethal, but usually, recovery occurs within a week (OIE Technical disease card, 2019). These effects of the disease make RVF an important illness in both an economical and human health aspect.

There are several interventions for reducing the possibility of an outbreak of RVF such as control of animal movements and control of animals at slaughterhouses. Reducing the hatching of mosquitoes through draining standing water or using insecticides lessens the risk of exposure to both animals and humans since it reduces the spread of the disease via vectors (OIE Technical disease card, 2019).

There are several vaccines available for animals but no specific treatment (OIE Technical disease card, 2019). The live-attenuated vaccines and the inactivated vaccines are available in endemic areas for limiting the disease (Ikegami, 2017).

Although, the different types of vaccines all have their pros and cons. The live attenuated vaccine with the Smithburn strain has, in some cases, been shown to cause fever spikes with following abortion in pregnant does and hepatic necrosis in young animals (Kamal, 2009). Animals vaccinated with the Smithburn strain also have the potential of spreading the disease to other susceptible animals and humans and there is a risk that this vaccine-strain reassembles with wild strains to cause a new virus strain (Kamal, 2009). The Smithburn vaccine, however, is effective and gives a long-lasting immunity, also protecting offspring through suckling, while being one of the cheapest to produce among vaccines for RVF (Lagerqvist, 2013).

The live attenuated Clone 13 and the newly developed MP-12 vaccines have been shown to rapidly protect vaccinated animals (Ikegami, 2017). Therefore, they are useful in protecting naïve animals in case of an outbreak. They have, however, been shown to cause malformations in foetuses when used in early pregnancy and therefore a safe vaccine for pregnant does is still

needed (Ikegami, 2017). Inactivated vaccines do not hold the same risk but require more frequent vaccination to be effective (OIE Rift Valley Fever, 2019). This, and the fact that these vaccines demand more workhours to be produced, make the inactivated vaccines more expensive and more inconvenient to use (Lagerquist, 2013).

Occurrence of disease in Zambia and nearby countries

Brucellosis

Brucella melitensis was in 2018 reported in Zambia's neighbouring country Tanzania and was suspected in Malawi. However, in Zambia and the rest of Zambia's neighbouring countries the OIE reports the disease to be absent (OIE Disease distribution maps, 2018). This does not mean that the pathogen is not present but simply that no outbreaks were reported during these years.

A few studies show evidence of the disease circulating in the country amongst both wild animals and domestic cattle (Muma *et al.*, 2006; Muma *et al.*, 2010; Ghirotti *et al.*, 1991). Amongst the wild ruminant species Kafue Lechwe, 21.6% had antibodies for brucellosis (Muma *et al.*, 2010). Ghirotti *et al.* showed that 28.5% of the cattle grazing in the same area in 1991 had antibodies to brucella. The authors hypothesized that the prevalence of disease could be because of wild ruminants and cattle grazing so close to one-another (Ghirotti *et al.*, 1991). In the area of Lochinvar and Blue Lagoon national parks, the seroprevalence of cattle was found to range between 14.1% and 28.1% in 2006 (Muma *et al.*, 2006). In this study, also goats and sheep were sampled, but none tested positive for brucella-specific antibodies (Muma *et al.*, 2006). In four districts of the Southern province of Zambia, the seroprevalence of brucellosis in cattle was determined to 20.7% in 2008 (Muma *et al.*, 2013). Another study performed a few years earlier found the prevalence of brucellosis, in the Southern and Lusaka provinces, to be 5.7% in total across the area. However, the areas where no vaccination occurred (Muma *et al.*, 2012).

CCHF

According to the OIE, CCHF has never been reported in Zambia. Surrounding countries have either never reported the disease or in 2018 the disease was absent (OIE Disease distribution maps, 2018). The Democratic Republic of Congo reported CCHF in 2015 but has since then been free from further outbreaks (OIE Disease distribution maps, 2018). Antibodies to the virus was found among cattle and goats in the southern parts of the Democratic Republic of Congo in 2017 (Sas *et al.*, 2017). The prevalence among cattle was 0.4% and in goats 5.9%. Sheep were also tested in the study, but none tested positive for CCHFV antibodies (Sas *et al.*, 2017).

Seroepidemiological studies show that the virus exists in both of Zambia's neighbouring countries Tanzania and Zimbabwe amongst cattle, sheep and goats. In Zimbabwe, antibodies to CCHFV has also been found amongst wild animals (Spengler *et al.*, 2017).

RVF

According to the OIE, it is unclear when Zambia last had an outbreak of RVF. During the year 2018, the disease was not reported (OIE Country information, 2019). However, according to a study from 1992, Zambia had an epizootic occurrence of RVF in cattle and sheep between the years 1982-86 (Davies *et al.*, 1985). The researchers analysed blood samples collected during these years in search for antibodies to RVFV. It was shown that 3-8% of the animals seroconverted every year, meaning that the disease was spreading to new animals. During this time there were also reports of clinical RVF in the districts of Lusaka, Mazabuka and Chisamba (Davies *et al.*, 1985). The Centers for Disease Control and Prevention (CDC) states that Zambia, in 2016, along with its neighbouring countries Tanzania, Mozambique, Zimbabwe, Botswana and Namibia, had substantial outbreaks of RVF and endemic disease. The rest of the neighbouring countries, Angola, Congo and Malawi, reported periodic isolation of the virus and few clinical cases (CDC, 2016).

In 2018, a study was performed in the districts of Nakonde and Mbala in Zambia, close to the border of Tanzania. In this study the seroprevalence of RVFV was found to be 2.2% in goats. The animals which tested positive were all located in the district of Nakonde (Linde, 2019). A study that focused on cattle in Zambia found the prevalence of antibodies to RVF to be 5.4% in 2018 (Saasa *et al.*, 2018).

Impact of disease

Brucellosis, RVF and CCHF are diseases which all affect small ruminants and cattle in different ways with signs ranging from mild fever to death. The diseases also cause a wide range of health issues among humans and this make them important to prevent and treat. But it is not only in the area of health that these pathogens influence the community. People who depend on small-scale farming to make a living are often dependent on their animals for food and income. Small ruminants play an especially important role in low-income society because they are cheaper to acquire than cattle. Loss of animals can cause a noticeable effect on a farmer's income (FAO, 2013). Both brucellosis and RVF cause abortions among animals. This slows down the recruiting of new animals to the herd, affecting the immediate number of animals available to sell for money, long-term breeding gene-material and animals available to slaughter for food. This can negatively affect the farmers income and in the long run make it impossible to pay for e.g. school fees, giving the diseases a social impact (Nitrandekura *et al.*, 2018)

Implementing animal health programmes in poor countries has been shown to help farmers who might otherwise be affected by trade-bans or reduced payment because of sick animals (Nin Pratt *et al.*, 2005), but the preventing measures can also have effects on a country. The use of vaccines may be necessary to stop a disease from spreading and this can be an expensive and laborious intervention. Preventing tick-borne diseases using acaricides may affect health, farming and other agricultural sectors due to the chemicals contaminating groundwater and environment. This shows that the diseases have a complex effect on a society (Rich and Perry, 2011).

MATERIAL AND METHODS

Samples

This project is a part of a PhD-project which studies infectious diseases of small ruminants in Zambia and Tanzania. The first part of this project took place in September and October of 2019 and involved collecting serum samples from domestic goats in Zambia. The samples were collected from three different districts in the central and southern parts of the country; Monze, Mazabuka and Chibombo, marked on the map below (Figure 1). These districts were selected because they did not border to another country and the local veterinary office agreed to be a part of the sampling process. The choice of districts was also discussed and selected in agreement with Dr Musso Munyeme at University of Zambia and Dr George Dautu at Central Veterinary Research Institute.



Figure 1. Map of the districts of Zambia. The selected study districts were Monze (outlined in black), Mazabuka (in green) and Chibombo (in red). Source: ontheworldmap.com.

The local district veterinary officers provided a list of villages in the districts and from each of these lists a random selection of ten villages per district was made. In the case of any of the first ten villages being unavailable, it was replaced with the next village on the randomized list. A village was deemed unavailable if it had less than four households with goats, less than four goats in every household or that it was impossible to reach the village by car.

For each village, four households were selected through snowball sampling, i.e. the first farmer directed us to the next farmer with goats and so forth. For each household, four goats, not under the age of four months, were selected for sampling. The limit of four months of age was set to try to exclude animals still carrying maternal antibodies. This resulted in 160 samples from 40 different households in each district, a total of 480 samples and 120 questionnaires. Unfortunately, two of the samples were lost in transport, resulting in 478 samples to analyse. The sample size was calculated at the website Epitools to achieve the number of samples that could help us determine a true prevalence of each disease. To calculate this the sensitivity and specificity of each ELISA test was used together with a margin of error of 5%, 95% confidence interval (CI) and an infinite population (Ausvet, 2019a).

The samples were kept in a cooling-bag during the day. In the end of the day, the serum was separated and transferred to a freezer as soon as possible, usually within 8 hours from sampling. Before sampling the next district, the samples were brought to the University of Zambia, Lusaka, and stored at -80 $^{\circ}$ C.

Laboratory analysis

For *Brucella*, a competitive ELISA from Boehringer-Ingelheim Svanova Diagnostics Uppsala, Sweden, was used called "*Brucella* c-ELISA antibody test". The test is designed to detect antibodies to *B. abortus*, *B. melitensis* and *B. suis*. The Brucella test kit has a sensitivity of 99.4% and a validity of 98.9% (Biancifiori *et al.*, 2000).

For both RVFV and CCHFV, ELISA-kits from ID-VET, Grabels, France, were used. The "ID Screen Rift Valley Fever Competition Multi-Species" with a sensitivity and specificity of 100% (Comtet *et al.*, 2010). For CCHF, the "ID-Screen CCHF Double Antigen Multi-species" was used, and this kit has a sensitivity of 98.9% and a specificity of 100% (Sas *et al.*, 2018). All tests were performed according to instructions from the manufacturers.

All samples were defrosted, and parts of each sample transferred to a plate in the same order that they were going to be placed on the ELISA plate. These preplates were stored a maximum of nine days in a refrigerator at 4°C until they were used for analysis.

Questionnaire

One or more members from each household were interviewed using a questionnaire (see Appendix 1). Many of the farmers did not speak English and therefore, to be able to do the questionnaire, an interpreter native to Zambia performed most of the interviews.

The serologic results were compared to answers to selected questions of the questionnaire to identify risk-factors for having serologically positive animals in the herd. The selected questions were as follows:

• How often are your goats in contact with sheep and/or goats from other herds?

- How often are your goats in contact with cattle from other herds?
- How often are your goats in contact with wild ruminants?
- How often do you dip and/or spray your goats for external parasites such as ticks and flies?
- When one or a few of your goats are sick, do you keep it/them separated from the rest of the herd?
- How often do you buy/barter or in any other way get new goats to your herd?

The answers to the questions in the questionnaire were tested to see if there was any association between different management routines and a positive result on serology. To be able to calculate the association, the answers to the questions had to be divided into two different categories. The three questions concerning contact with other sheep or goat herds, cattle herds or wild ruminants were divided into the answers "never" and "once a week". The answers that were once a week or more frequent were included in the category "once a week" and the answers that meant the goats had contact with other animals less frequently than once a week were included in the category "never". For the question on how often the farmers dipped or sprayed their animals the answers were divided into "never" and "once every two weeks". On the question of isolation, the answers to how often the farmers bought or acquired new goats was divided into "never" and "every six months".

Statistics

True prevalence was calculated at the website Epitools using the Clopper pearson exact test (Ausvet, 2019b). The association between questionnaire answers and serological results was analysed with Fisher's exact test in all cases but two that were analysed with chi2-test. The change of test used was because all but the two analysed with chi2-test had five or less individuals in one of the categorises.

RESULTS

The sampled herds ranged from six individuals up to 185, with many of the herds consisting of less than 50 individuals. The distribution of herd size is shown in Figure 2 below.



Figure 2. Sizes of the sampled goat herds divided in groups of ten individuals.

Serology

Brucellosis

Out of 478 samples, 27 animals (5.6%) tested positive for brucellosis (Table 1). The samples that tested positive were all tested a second time and came up positive also in the second ELISA. These samples were collected from 15 different households, with most of the positive villages in the district of Mazabuka (Table 2). When counting the household as positive only when at least two goats were seropositive, the number of positive households was reduced to five, all located in Mazabuka district (Table 3).

Disease	Collected	Positive	Prevalence	True	Households	Households
	samples	samples	(%)	prevalence	with at least	with at least
	(n)	(n)		(% with	1 positive	2 positive
				95% CI)	goat (n)	goats (n)
Brucellosis	478	27	5.6	4,6 (2.7-	15	5
				7.1)		
CCHF	478	25	5.2	5.2 (3.5-	19	4
				7.6)		
RVF	478	0	0	0	0	0

Table 1. Positive samples and prevalence of each disease in all districts combined

In Figure 3, negative and positive individuals for brucellosis are distributed according to age. The figure shows a rather similar distribution of age between animals tested negative and positive to antibodies for brucellosis. Fisher's exact test showed no association between age and positive or negative results.



Figure 3. Distribution of age for goats positive and negative for antibodies to brucellosis. Age is given in years.

CCHF

Of the goats sampled, 25 from 19 of the visited households tested positive to CCHFV-antibodies. If a herd was counted as positive only when two animals in the household was positive, there were 4 positive households (Table 1). Many of the households with one positive individual were in the district of Mazabuka (Table 2). When changing the criterion for positive household to two positive individuals the remaining households were in Monze and Mazabuka (Table 3).

Table 2. Number of positive households and prevalence per district, at least one positive goat in a household

District	Brucellosis		CC	CHF
	Positive	Prevalence (%)	Positive	Prevalence (%)
	households (n)		households (n)	
Monze	2	5.0 %	4	10.0 %
Mazabuka	10	25.0 %	14	35.0 %
Chibombo	3	7.5 %	1	2.5 %
Total	15	12.5 %	19	15.8 %

District	Brucellosis		CC	CHF
	Positive Prevalence (Positive	Prevalence (%)
	households (n)		households (n)	
Monze	0	0	1	2.5 %
Mazabuka	5	12.5 %	3	7.5 %
Chibombo	0	0	0	0
Total	5	4.2 %	4	3.3 %

Table 3. Number of positive households and prevalence per district, at least 2 positive goats in a household

Figure 4 shows the distribution of age between the CCHFV antibody negative and positive goats. No association could be found between age and positive or negative results (Fisher's exact test; p-value = 0.177).



Figure 4. Distribution of age for goats positive and negative for antibodies to CCHF. Age is given in years.

RVF

Two samples of the 478 collected samples gave a doubtful result for RVFV antibodies. These samples were run twice to confirm the result and one remained doubtful, while the other tested negative in the second run. The rest of the samples collected tested negative for RVFV antibodies. Therefore, these results were not further analysed.

Questionnaire

At first the association was calculated with the households being counted as positive only if they had two or more goats tested as positive in the herd. This gave an association between the goats having contact with cattle from other herds and being positive to CCHFV antibodies with a p-value of 0.026. The other factors showed no association with the serology results (Table 4).

	COUE	D	Coot off
	CCHF	Brucellosis	Cut off
Contact with	0.42	0.12	Once a week
sheep/goat herds			
Contact with cattle	0.026	0.28	Once a week
herds			
Contact with wild	1	1	Once a week
ruminants			
Frequency tick	0.32	0.39	Once every two
treatment			weeks
Isolation when sick	1	1	Yes/no
Frequency buying	0.32	0.32	Every six months
new goats			

Table 4. *P-values for association between serological status at herd level and questionnaire data calculated with Fishers exact test. An antibody-positive herd had at least two positive goats*

Among the sampled goat herds 94 of the herds had contact with cattle from other herds at least once a week while 24 had contact with other cattle more rarely. Two of the households could not respond to this question. See also Figure 5.



Figure 5. Frequency of the sampled goat herds encountering cattle from other households.

There were also many of the sampled goat herds that lived on a farm that also kept cattle. Only five of the sampled households responded that they did not have cattle, two did not answer the question (Figure 6).



Figure 6. Number of households that had both goats and cattle are shown in the category "yes".

To see if there was any difference in association with other cut offs in the categorization of the answers, all cut offs except the ones for isolation were changed and all results recalculated. The answers for questions regarding contact with other animals were all divided into "never" and "at least every six months". Frequency of tick treatment was divided into "never" and "at least once a month". For the question about buying new goats the answers were divided into "never" and "once every six months". All p-values for association are showed in Table 5. The only association found was between CCHF positive serology and contact with other cattle herds.

	CCHF	Brucellosis	Cut off
Contact with	0.4	0.11	Every six months
sheep/goat herds			
Contact with cattle	0.027	0.28	Every six months
herds			
Contact with wild	1	1	Every six months
ruminants			
Frequency tick	1	1	Once a month
treatment			
Frequency buying	0.32	0.32	Every six months
new goats			

Table 5. *P-values for association between serological status at herd level and questionnaire data calculated with Fishers exact test. An antibody-positive herd had at least two positive goats*

The households were also counted as positive if one individual in their herd tested positive. This was done to see if a higher prevalence would affect the results. No association was found between the management routines and the serology results in these calculations.

DISCUSSION

Serology

In this study, the true prevalence of positive serological results to CCHFV antibodies was found to be 5.2% and to brucellosis 4.6%. No antibodies to RVF could be confirmed. At the same time as this study was performed, another part of the PhD-project was taking place in four districts of Zambia close to the borders to other countries; Chavuma bordering Angola, Chililalombwe bordering the Democratic Republic of Congo, Vubwi bordering to both Mozambique and Malawi and Siavonga bordering Zimbabwe. This part of the project also investigated the prevalence for brucellosis, CCHF and RVF among goats. Those results are at the time of writing still preliminary, but shows a prevalence to brucellosis ranging between 1.7 – 4.2%, except for the Siavonga district that had a preliminary prevalence of 41.7% (Personal communication with Sara Lysholm). To CCHF, the outer districts had a prevalence ranging between 0% - 4.2% and a prevalence of RVF between 0% - 0.8%. Those results are close to the ones found in the central districts in this study.

Previous studies have shown the prevalence of brucellosis to be higher in Zambian cattle, ranging from 14.1% - 28.5% between the years 1991 - 2008 (Ghirotti *et al.*, 1991; Muma *et al.*, 2006; Muma *et al.*, 2013). In one study, the prevalence was closer to the one found in this study with 5.7% in the Southern and Lusaka province (Muma *et al.*, 2012).

Since larger herd size and higher parity number is a risk factor for higher seroprevalence, one possible explanation for the difference in seroprevalence with the above-mentioned studies is that cattle are kept in larger herds than goats and are kept for a longer period before slaughter. The difference in management between cattle and goats might be because cattle are considered more valuable and therefore also an investment for the future.

CCHF is circulating in Zambia's neighbouring countries but the seroprevalence of the disease has not been thoroughly investigated inside Zambia. Hence there are not many other studies to compare our results with.

According to the CDC, RVF is circulating in Zambia and its neighbouring countries. RVF has been reported in goats in the district of Nakonde with a seroprevalence of 2.2% (Linde, 2019). Zambian cattle have been tested with a seroprevalence of 5.4% in 2018 (Saasa *et al.*, 2018). Therefore, it was unexpected to find no seropositive goats in the visited households. Similarly, in the districts visited by Sara Lysholm the prevalence for RVF was low. This could be attributed to the fact that most of the animals we sampled were relatively young, most of the animals were between one and three years old. If this was during an inter-epidemic episode, many of these animals might never have been in contact with the virus because no mass hatching of the vector mosquitoes had occurred during their lifetime.

There could also be a geographical difference in Zambia to where RVF is more common. Since Zambia's neighbouring countries also report occurrence of disease it could be that the disease

is spreading from these countries and that they have more active vectors. This would make the disease more common in districts bordering other countries.

The prevalence can be skewed due to different reasons. The first being our selection of animals. When collecting the samples, the goats were not selected in a randomised way, but instead based on our ability to catch them. This could mean that the sick animals were easier to catch and thus making the prevalence to rise. Sometimes the farmers had already released their animals out into the field before we arrived. They then had to gather them up and sometimes they only found parts of the herd, bringing only as many goats as was required for sampling. This could mean that we did not get four animals that were representative to the entire herd.

Risk factor analysis

In the results found during this study, only contact with other cattle herds showed an association with being serologically positive to CCHF antibodies. This could be because cattle carry more ticks that spread the disease. This is however unlikely to be the reason since most of the goat herds also live with cattle in the same household. Another theory is that these areas have a denser population of animals making them come in closer contact than in districts with primarily sheep or goat herds. The association could also be because of an external factor such as environmental differences. In the areas where goats and cattle often graze together, the environment could be more tick friendly and thus there are more vectors to spread the disease.

With respect to CCHF it was expected to find an association with the frequency of tick treatment, such as dipping or spraying, since the virus mainly spread through its vector. This was not the case and no association could be found even if the cut off of the answers or when the definition of a positive household were changed.

Brucellosis has previously been studied regarding risk factors. It has been shown that larger herds, higher age and higher parity number are all risk factors for contracting the disease (Alhamada *et al.*, 2017; Tegegn *et al.*, 2016). In this study there was no association between age and seroprevalence. Herd size was not investigated as a risk factor in this study.

A study of Zambian cattle showed that exposure to wild ruminants was a risk factor to brucellosis (Muma *et al.*, 2007). This study could not find that association. The difference could be in how the data was described. In the study by Muma *et al.*, the cattle were divided into groups who had or did not have contact with wild ruminants, while in this study the data was divided by more or less frequent contact than once a week or every six months. Changing those limits divides the data into new groups that may affect the calculated association.

When performing an interview through an interpreter there is always a risk of the answers being altered. Through the translation, small nuances in the answers might be lost. In this case, the answers have been translated from the respondent's first language to English by our interpreter who speaks both languages, written down and then interpreted again by someone who speaks Swedish and English and divided into different categories. To reduce the risk of mistakes in the interpretation, our interpreter was local to Zambia and therefore had a deeper understanding of

the culture from which the respondents came, making it easier to pick up subtle differences and interpret gestures. Another way to reduce mistakes was to have categorial answers already in the questionnaire thus making it harder to misunderstand.

POPULAR SCIENCE SUMMARY

Small ruminants, such as sheep and goats, are important to farmers in low-income countries because they are usually cheaper to acquire and constitute an important source of income and food. Losing these animals because of disease can greatly affect the owner and his/her family. When losing income, it lessens the children's opportunity to go to school or the family's ability to buy clothes and other necessities.

The diseases focused on in this study are brucellosis, Crimean-Congo haemorrhagic fever (CCHF) and Rift Valley fever (RVF). All these diseases infect animals and humans alike and can spread between them when in close contact with body fluids or when consuming untreated milk or meat from infected animals. Brucellosis and RVF cause abortions in animals and this also lowers the production and further expansion of the farmers herd.

This study had two goals. The first was to find animals with antibodies to the selected diseases. If the animal has antibodies it means that it has been exposed to the disease-causing microbe at some point. The existence of antibodies is a good indication that the disease is circulating among animals in the area.

The second goal of the study was to see if any management routines could be a risk factor for contracting the disease. To be able to do this, all farmers were asked questions according to a questionnaire involving their management routines and details about their animals.

The study started in September of 2019 by collecting blood samples from goats in three different districts in Zambia. Monze and Mazabuka in the Southern province and the district of Chibombo in the Central province. In each district, ten villages were selected and in every village four farmers with at least four goats were visited. This provided 480 blood samples and 120 answered questionnaires for analysis.

When analysing the results, 2.7% of the animals had antibodies to brucellosis, 5.2% had antibodies to CCHF, but no antibodies to RVF could be found. These numbers are similar to what other studies have found in the area among goats or cattle, except that antibodies to RVFV have been found previously in Zambia.

The questionnaires were summarised and compared to the results from the blood samples to search for associations between management routines and the antibody status of the goats. An association was found between having antibodies for CCHF and that the goats were in contact with cattle from other herds at least once every six months.

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Department of Clinical Sciences

Questionnaire

2019

QUESTIONNAIRE SMALL LIVESTOCK FARMERS

ID: 2019-ZM-

DATE: ___/__/2019

CHECK IF:

LOCATION

District:	Town/Village:	GPS: Latitude
		GPS: Longitude

Interview language	Respondents first language(s)

1. MANAGEMENT ROUTINES

1.1) What grazing system are you utilizing? OBS: Remember to ask the farmer to specify the different grazing systems for ALL seasons

Grazing system	Check	Comment
	the ones	E.g. <u>season</u> , animal type (kids, pregnant mothers)
	that	
	apply	
Communal grazing		
Fenced grazing		
Tethering		
Herding		
Zero-grazing/Cut-and-carry		
Other, please specify;		

1.2a) How often are your goats in contact with sheep and/or goats from other herds?

If the farmer uses several grazing systems, e.g. communal grazing during the dry period and tethering during the rainy season; remember to ask about the contact patterns of both grazing system

FREQUENCY	Check the
	one that
	applies
Daily	
At least once a week	
At least once a month	
At least once every 6	
months	
At least once a year	
More rarely	
Never	
I do not know	

1.2b) ASK only if respondent did not answer NEVER on 1.2a):

Does this vary over the year? If yes, how?

For example; more contact during dry season etc

1.2c) ASK only if respondent did not answer never on 1.2a)

Please describe this contact

For example grazing together, grazing on the same field but not together etc

1.3a) How often are your goats in contact with cattle from other herds?

If the farmer uses several grazing systems, e.g. communal grazing during the dry period and tethering during the rainy season; remember to ask about the contact patterns of both grazing system

FREQUENCY	Check the one that applies
Daily	
At least once a week	
At least once a month	
At least once every 6 months	
At least once a year	
More rarely	
Never	
I do not know	

1.3b) ASK only if respondent did not answer never on 1.3a):

Does this vary over the year? If yes, how?

(For example; more contact during dry season etc)

1.3c) ASK only if respondent did not answer never on 1.3a)

Please describe this contact

(For example grazing together, grazing on the same field but not together etc...)

1.4a) How often are your goats in contact with wild ruminants?

If the farmer uses several grazing systems, e.g. communal grazing during the dry period and tethering during the rainy season; remember to ask about the contact patterns of both grazing system

FREQUENCY	Check the one that applies
Daily	
At least once a week	
At least once a month	
At least once every 6	
months	
At least once a year	
More rarely	
Never	
I do not know	

1.4b) ASK only if respondent did not answer never on 1.4a)

What species of wild ruminant(s)?

If the respondent does not know the English name, write the local name

1.4c) ASK only if respondent did not answer never on 1.4a)

Does this vary over the year? If yes, how?

(For example; more contact during dry season etc)

1.4d) <u>ASK only if respondent did not answer never on 1.4a</u>) Please describe this contact

(For example grazing together, grazing on the same field but not together etc...)

2. MEDICINES

2.1a) How often do you dip and/or spray your goats for external parasites such as ticks and flies?

FREQUENCY	Check the ones that apply	Comments E.g. in the rain season at least once a week, in the dry season at least once a month etc
At least once a week		
At least once every two weeks		
At least once a month		
At least once every three months		
At least once every six months		
At least once a year		
More rarely		
I never dip or spray them		

2.1b) <u>ASK ONLY IF THE RESPONDENT DID NOT ANSWER NEVER ON 2.1a)</u> When do you decide that it is time to dip/spray your goats?

2.2a) How often do you deworm your goats?

FREQUENCY	Check the ones that apply	Comments E.g. in the rain season at least once a week, in the dry season at least once a month etc
At least once a week		
At least once every two weeks		
At least once a month		
At least once every three months		
At least once every six months		
At least once a year		
More rarely		
I never deworm them		

2.2b) <u>Ask ONLY if the farmer did not answer NEVER on question 2.2.a</u>) How do you decide that it is time to deworm the goats?

2.3a) When was the last time you treated your goats with a medicine that was not a dewormer or a spray/dip for external parasites?

FREQUENCY	Check the one that applies
This week	
This month	
These last six months	
This year	
More than one year ago	
I have never treated my goats for	
anything other than deworming	
and tick spray	

2.3b) Ask only IF YES to 2.1a) What drug did you use at the last time?

NAME and TYPE of drug: _____

2.3c) Ask only IF YES to 2.1a) What symptoms/disease did the goats have at this time?

2.4a) Do you vaccinate your goats?

YES	NO

2.4b) Ask ONLY if the respondent answered YES to 2.4.a

For what diseases do you vaccinate your goats?

2.4c) Ask ONLY if the respondent answered YES to 2.4.a

When was the last time you vaccinated the goats?

2.4d) Ask ONLY if the respondent answered YES to 2.4.a

How often do you vaccinate them?

2.5) When one or a few of your goats are sick, do you keep it/them separated from the rest of the herd?

YES, during	YES, during	YES, both day	NO
daytime	night time	and night time	

3. TRADE

3.1a) How often do you <u>buy</u>/barter or in any other way get new goats to your herd?

This question is about all ways to get new animals to the herd except through birth from animals that are already part of the herd

FREQUENCY	Check the one that
	applies
At least once a week	
At least once a month	
At least once every six	
months	
At least once a year	
At least once every	
two years	
More rarely	
I have never	
bought/bartered a	
goat	

3.1b) ASK ONLY if the respondent did not answer "I have never..." on question 3.1a

What kind of people do you buy/barter or in any other way get new goats to your herd from?

Write	1	or	0	and	rank	the	alternatives
	-	U 1	~			cric	ancennachtes

TYPE OF PEOPLE	Check the ones that apply	RANKING 1= most common, 2= second most common etc
Farmers		
Traders		
Other; specify;		

3.1c) ASK ONLY if the respondent did not answer "I have never..." on question 3.1a. Where are these goats from?

Write 1 or 0 and rank the alternatives

LOCATION	Check the ones that apply	RANKING 1= most common, 2=
From my village		
From other villages in my district		
From other districts If YES, specify;		
From markets If YES; Please specify location of the market(s)		
Other, please specify;		

3.2) Have you ever bought or received one or several goats from other countries?

YES	NO
Which countries?	
 When was the last time you bought or received a goat from another country? 	

3.3) Are you aware of farmers in your community who are buying or receiving sheep and/or goats from other countries?

YES	NO
If YES; Which countries?	

3.4) After acquiring new goats, do you let them mix with your original herd immediately?

YES	NO
	If NO; what do you do? For example; keep them separated for a day,
	deworms them then release them with the rest etc

3.5) When do you decide to sell goats?

3.6) How do you decide which goats to sell?

3.7a) How often, approximately, do you sell goats?

FREQUENCY	Check the one that applies
At least once a week	
At least once a month	
At least every six months	
At least once a year	
At least once every two years	
More rarely	
I have never sold goats	

3.7b) ASK ONLY if the respondent did not answer "I have never..." on question 3.7a. What kind of people do you sell your goats to?

	Write 1 d	or 0 and	rank the	alternatives
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TYPE OF PEOPLE	Check the ones that apply	Ranking
		1= most common,
		2=second most etc
Farmers		
Traders		
Home consumers		
(People buying for		
home consumption)		
At markets (to		
people who buy at		
markets)		
Restaurants		
Slaughterhouse		
Other		
If YES; please		
specify;		

3.7c) ASK ONLY if the respondent did not answer "I have never..." on question 3.7a. To where do you sell the goats?

LOCATION	Check the ones that	RANKING
	apply	1= most common, 2=
		second most common
Within my village		
Within my district		
To other districts		
If YES, specify		
To other countries		
If YES, specify		
Other, please specify;		

3.8) Have you ever sold goats to other countries?

YES	NO
If YES; Which countries?	

3.9) Are you aware of farmers in your community who are selling goats to other countries?

YES	NO
If YES; Which countries?	

3.10) Which diseases is it OK for a goat to have and it can still be sold?

First ask the question as it is and write the answer in the free space, then probe for the alternatives below and note down the answers in the table

Probe for;	1=YES
	0=NO
Runny eyes and nose	
Coughing	
Diarrhea	
Abortion	
Other, please specify;	

3.11) What diseases would you say that it is OK for the goat to have and you would still buy it?

First ask the question as it is and write the answer in the free space, then probe for the alternatives below and note down the answers in the table

Probe for;	1=YES
	0=NO
Runny eyes and nose	
Coughing	
Diarrhea	
Abortion	
Other, please specify;	

3.12) Are there any risks with trading sick animals?

Answer	Check the one that applies
Yes	If YES; what are the risks:
No	
l do not know	

4. ANIMAL HEALTH

4.1) What signs of disease did you observe in your goats, in the last 12 months?

1. Ask what signs of disease the farmer experiences in his/her animals and indicate below

- 2. Ask farmer if the occurrence of the disease varies over the year or if it occurs at a constant rate
- 3. Ask the farmer to rank the diseases from the most common disease to the least common.
- 4. Ask what the farmer does when he or she experiences this symptom/disease

Disease/Symptom	YES = 1, NO = 0	Rank from most common (1) to least common (Top 5)	Seasonality	What do you do when you experience this?
4.1a.Diarrhea				
4.1b.Coughing				
4.1c.Abortion				
4.1d.Dying kids				
4.1e.Sudden death (dying suddenly within 24 hours of showing symptoms or not showing symptoms at all)				
4.1f.Runny eyes and nose				

4.2) What disease would you say have the highest impact on you as a farmer? Why this disease?

4.3) Do you ask anyone for help when your goats are sick and if YES, who do you ask?

ТҮРЕ	CHECK THE ONE THAT	RANK (1= most
	APPLIES	common, 2=second
		most common etc)
I do not ask for help		
Other farmers		
Vet shop/Agri shop		
Veterinary personnel		
(vet, vet assistant,		
livestock assistant etc)		
Pharmacy		
Other		

5. ANIMAL SPECIES IN HOUSEHOLD

6.1) Are the goats housed together with any other species? If Yes, which species?

YES	NO
If YES; Please specify species;	

6.2) What species other than goats do the household have?

Type of species	Present in	Comments
	household?	
	1=YES	
	0=NO	
Sheep		
Cattle		
Pigs		
Horses/Donkeys		
Poultry		
Other;		

6.3) Details of goats owned

	Adult males	Adult females	Kids/Lambs
Number			
Breeds			

6.4) Details of sheep owned

	Adult males	Adult females	Kids/Lambs
Number			
Breeds			

6. INTERVIEW DETAILS

7.1) Interviewee role in the household and in taking care of the goats

7.2) How long has your household been keeping goats?

7.4) Gender

Male	Female

7.4) Farmers first name and telephone number (optional for farmer – this is so that we can give them feedback about the results)

Do you have any questions for me/us?