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Crossing the line – Tracking transboundary diseases in trade and across international borders in Zambia and Tanzania

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Cover image: Goats and cattle in a pen in Southern Province, Zambia
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Abstract

Transboundary diseases in small ruminants constitute a threat to health, livelihoods and society in Zambia and Tanzania. Animal trade and movement, both of which tend to increase close to international borders, are known drivers of disease spread. This thesis was guided by three overarching aims. The first aim was to investigate the impact of trade and border proximity on the seroprevalence of peste des petits ruminants (PPR), contagious caprine pleuropneumonia (CCPP), foot and mouth disease (FMD), sheeppox and goatpox (SGP), Rift Valley fever (RVF), Crimean-Congo haemorrhagic fever (CCHF) and brucellosis in sheep and goats in Zambia and in the Tanzania-Zambia border region. The second aim was to investigate how the perceptions and practices of Zambian small ruminant traders and slaughterhouse workers influence the risk of disease spread. The third aim was to investigate the seropositivity rate of brucellosis, Q-fever and RVF in sheep and goats in Zambia's two largest small ruminant markets, and risks for exposure to these and other zoonotic diseases at a market slaughterhouse. To meet these aims, seroepidemiology was combined with semi-structured interviews, focus group discussions and observations. The results indicated that most of the surveyed pathogens are circulating in parts of Zambia and Tanzania, and proximity to international borders was generally associated with reduced seroprevalence. While the impact of trade on seroprevalence varied, the perceptions and practices of small ruminant traders pose risks of disease dissemination through trade. The presence of zoonotic pathogens at small ruminant markets in Zambia coupled with insanitary procedures at slaughter represent potential threats to public health. In conclusion, small ruminant transboundary diseases pose serious risks. Informed control strategies adapted to the local context could mitigate these risks, improving the health and welfare of animals and humans alike.

Keywords: sheep, goat, transboundary disease, zoonosis, international border, trade, markets, epidemiology, semi-structured interviews, focus group discussions

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Sammanfattning

Smittsamma får- och getsjukdomar utgör hot mot hälsa, försörjning och samhällsutveckling i Zambia och Tanzania. Förflyttning av och handel med djur kan bidra till sjukdomsspridning, och sådana aktiviteter tenderar att öka frekvens i närheten av landsgränser. Denna avhandling har tre övergripande syften. Det första syftet var att undersöka hur handel och gränsnärhet inverkar på seroprevalensen av peste des petits ruminants (PPR), smittsam pleuropneumoni (CCPP), mul- och klövsjuka (FMD), får- och getkoppor (SGP), Rift Valley feber (RVF), Krim-Kongo blödarfeber (CCHF) och brucellos hos får och getter i Zambia och i den tanzaniska gränsregionen mot Zambia. Det andra syftet var att utreda hur zambiska djurhandlares och slaktares resonemang och agerande inverkar på risken för sjukdomsspridning. Det tredje syftet var att undersöka frekvensen av seropositivitet för brucellos, Q-feber och RVF hos får och getter på de två största marknaderna för små idisslare i Zambia, samt exponeringsrisker för dessa och andra zoonotiska sjukdomar på ett marknadsslakteri. För att uppnå dessa syften kombinerades sero-epidemiologi med semistrukturerade intervjuer, fokusgruppsdiskussioner och observationer. Resultaten indikerar att de flesta av de undersökta smittämnena cirkulerar i delar av såväl Zambia som Tanzania, och närhet till internationella gränser var generellt associerat med lägre seroprevalens. Djurhandelns inverkan på seroprevalensen varierade, men zambiska djurhandlares resonemang och agerande visades kunna bidra till ökad sjukdomsspridning. Förekomsten av zoonotiska smittämnen på marknader för små idisslare, i kombination med hälsovådliga slaktprocedurer, utgör hot mot zambisk folkhälsa. Sammanfattningsvis utgör infektionssjukdomar som drabbar får och getter allvarliga hot. För att minska dessa hot krävs välunderbyggda kontrollåtgärder som är anpassade efter lokala förhållanden, vilket kan leda till förbättrad hälsa och välfärd hos såväl djur som människor.

Nyckelord: får, get, infektionssjukdom, zoonos, internationell gräns, handel, marknad, epidemiologi, semi-strukturerad intervju, fokusgruppsdiskussion

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Dedication

To my parents.

I love you more than I love goats, and you know how I feel about goats

James Patterson

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Lysholm, S., Lindahl, J., Munyeme, M., Misinzo, G., Mathew, C., Alvåsen, K., Dautu, G., Linde, S., Mitternacht, L., Olovsson, E., Wilén, E., Berg, M., Johansson Wensman, J. Crossing the line: Seroprevalence and risk factors for transboundary diseases along the Tanzania-Zambia border *(Submitted)*

II. Lysholm, S., Lindahl, J., Dautu, G., Johansson, E., Karlsson Bergkvist, P., Munyeme, M., Johansson Wensman, J. Seroepidemiology of transboundary pathogens in goats in Zambia with reference to trade and border proximity *(Submitted)*

III. Lysholm, S., Johansson Wensman, J., Munyeme, M., Fischer, K. (2020). Perceptions and practices among Zambian sheep and goat traders concerning small ruminant health and disease. PLOS ONE, 15(6), e0233611

IV. Lysholm, S., Fischer, K., Lindahl, J., Munyeme, M., Johansson Wensman, J. (2021). Seropositivity rates of zoonotic pathogens in small ruminants and associated public health risks at informal urban markets in Zambia Acta Tropica, In Press. 10.1016/j.actatropica.2021.106217

The contribution of Sara Lysholm to the papers included in this thesis was as follows:

I. Designed and planned the study together with the co-authors. Facilitated and coordinated all steps in the data collection and laboratory analysis process, and performed parts of the sample collection, questionnaire interviews and laboratory work. Organised and analysed the data. Drafted the manuscript with input from the co-authors and handled correspondence with the journal.

II. Designed and planned the study together with the co-authors. Facilitated and coordinated all steps in the data collection and laboratory analysis process. Performed more than 50% of the sample collection, questionnaire interviews and laboratory work. Organised and analysed the data with help from JL. Drafted the manuscript with input from the co-authors and handled correspondence with the journal.

III. Designed and planned the study together with the co-authors. Performed the interviews and observation sessions. Organised and analysed the data with help from KF. Drafted the manuscript with input from the co-authors and handled correspondence with the journal.

IV. Designed and planned the study together with the co-authors. Performed the sample collection, interviews, focus group discussions and observation sessions, as well as the laboratory work. Organised and analysed the data. Drafted the manuscript with input from the co-authors and handled correspondence with the journal.

Abbreviations

AIC	Akaike information criterion
ASF	African swine fever
CCHF	Crimean-Congo haemorrhagic fever
CCHFV	Crimean-Congo haemorrhagic fever virus
CCPP	Contagious caprine pleuropneumonia
cELISA	Competitive enzyme-linked immunosorbent assay
iELISA	Indirect enzyme-linked immunosorbent assay
CFR	Case fatality rate
CI	Confidence interval
DNA	Deoxyribonucleic acid
DRC	Democratic Republic of the Congo
FAO	Food and Agriculture Organisation
FMD	Foot and mouth disease
FMDV	Foot and mouth disease virus
FGD	Focus group discussion
GDP	Gross domestic product
GTP	Goatpox
GTPV	Goatpox virus
LDC	Least Developed Country
LR-test	Likelihood ratio test
LSD	Lumpy skin disease
LSDV	Lumpy skin disease virus
Mccp	*Mycoplasma capricolum* subspecies *capripneumoniae*
MeV	Measles virus
NZD	Neglected zoonotic disease
OIE	World Organisation for Animal Health

OR	Odds ratio
PPR	Peste des petits ruminants
PPRV	Peste-des-petits-ruminants virus
RNA	Ribonucleic acid
RPV	Rinderpest virus
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
SAT	South African Territories
SDG	Sustainable Development Goal
SGP	Sheeppox and goatpox
SGPV	Sheeppox and goatpox virus
SPP	Sheeppox
SPPV	Sheeppox virus
SLAZ	Small Livestock Association of Zambia
SSA	Sub-Saharan Africa
SSI	Semi-structured interview
TAD	Transboundary animal disease
VIF	Variance inflation factor
VNT	Virus neutralisation test
WHO	World Health Organisation
UK	United Kingdom
UN	United Nations
USD	U.S. dollar

1. Introduction

The role agriculture plays in the fight against poverty and hunger is well-recognized (Timmer 2005; Diao *et al.* 2010; Fan & Cho 2021). One of the fastest growing agricultural subsectors in low- and lower-middle-income countries is the livestock sector (Blench *et al.* 2003; Pica *et al.* 2008; FAO 2011; Enahoro *et al.* 2019), and its contribution to GDP is growing rapidly (Thornton 2010). However, the sector has historically been neglected by governments, aid organisations and in research, and it has only received a fraction of the funding that has been allocated to crop farming (Kruska *et al.* 2003). Approximately one billion people living on less than 2 USD per day are partially or completely dependent on livestock, which equates to approximately two thirds of the world's rural poor and one third of the urban poor. In Sub-Saharan Africa (SSA), there are approximately 300 million farmers who, to varying extents, depend on livestock for their livelihoods (Grace *et al.* 2012). These farmers produce a large share of the crops and livestock products, and hence are vital to food production in the region (Otte *et al.* 2012).

Historically, the focus of most livestock-related research activities and investment strategies has been on cattle. However, since cattle are often kept by more affluent farmers, this species may be less suitable if the goal is to alleviate poverty and hunger. Small ruminants (sheep and goats), however, are generally more common among poorer households (Perry *et al.* 2002; Pica-Ciamarra *et al.* 2011). These species play multiple important roles for millions of livestock farmers, for example as a source of income and food and as a provider of livelihood security and resilience. In addition, they generate income for several other value chain members, such as traders and slaughterhouse workers, and constitute a source of animal-derived food for many more consumers (FAO & OIE 2015).

As a result of the importance of small ruminants to a large proportion of the poor, infectious diseases affecting these species can have detrimental effects on the livelihoods of millions of rural and urban citizens (Heffernan 2009; Pradère 2014). Such diseases can result in poor production, for example through limited growth, reduced milk yields, abortions and mortalities (Perry & Grace 2009), and the effects can be seen both in individual animals and in whole herds or villages. As many of these diseases are zoonotic and transmissible to humans, for example at slaughter or through consumption of animal products, they can also have a serious impact on public health (WHO 2012). Human activities play important roles in the dissemination of animal diseases (Hidano *et al.* 2018). Aspects such as trade and animal movements are well-known drivers of disease spread (Sherman 2011), both of which tend to increase close to international borders (Perry & Hedger 1984; Di Nardo *et al.* 2011; Picado *et al.* 2011; Hamoonga *et al.* 2014; Sinkala *et al.* 2014a; Allepuz *et al.* 2015). Therefore, animal markets and border crossings can play important roles in the epidemiology of small ruminant diseases.

This thesis combined seroepidemiology and qualitative data strategies to address current knowledge gaps and expand the knowledge basis of the circulation of selected small ruminant diseases in farms and markets in Zambia and in the Tanzania-Zambia border region. The insights from this work will not only benefit the small ruminant population, but also the health and welfare of the millions of people who depend on them for their livelihoods.

1.1 The importance of small ruminants to poor peoples' livelihoods in Sub-Saharan Africa

Small ruminants are often termed as "the poor man's cow" because of their importance to poor livestock farmers (Omondi *et al.* 2008). They are relatively cheap to purchase and require comparatively little investment and management (Peacock 2005; FAO 2013; de Haan *et al.* 2015). As they reach sexual maturity at a young age, have short gestational periods and are able to give birth to multiple young, herd size is comparatively easy to maintain, despite the occurrence of adverse events such as droughts (Peacock 2005; de Haan *et al.* 2015). Sheep and goats are versatile species that can be incorporated in multiple farming systems and livelihood strategies. Small ruminants can maintain their body weight even with poorer feedstuff, and they generally fend better for themselves during extreme climatic events. Compared with cattle, goats have a wider range of foraging behaviour which enables them to survive on grass and foliage that would be unsuitable for cattle (Lovelace *et al.* 1993; Ahmadu & Lovelace 2002; Joy *et al.* 2020). They can be kept in areas where crop production is difficult, e.g. due to erratic or insufficient rainfall, high temperatures and poor land quality (Peacock 2005; Degen 2007; Joy *et al.* 2020). In addition, small ruminants often play important socio-economic roles, for example as gifts to newly-weds, in different traditional ceremonies, and they can be used to settle disputes within the community (Peacock 2005; Orskov 2011; Ejlertsen *et al.* 2013; de Haan *et al.* 2015).

An important benefit of keeping small ruminants is the ability to earn an income through sales, either of live animals or animal products such as meat, milk, skins, hides, wool or fibres (Peacock 2005; de Haan *et al.* 2015; FAO & OIE 2016). As small ruminants are often sold locally, the market base is large and easily accessible. The trade and market systems are often informal, with comparatively few inherent standards and legal requirements, making small ruminants easier to sell than many other livestock species (de Haan *et al.* 2015). Therefore, sheep and goats are major sources of income and contributors to the cash flow in farmer households (Otte & Chilonda 2002; de Haan *et al.* 2015; Kaumbata *et al.* 2020). Thanks to their fast reproductive rate, small ruminants can be used to both generate and accumulate assets (de Haan *et al.* 2015). In the absence of financial institutions in many rural areas in low- and lower-middle-income countries, small ruminants are frequently used as a means of storing wealth. Keeping small ruminants can therefore considerably improve a household's resilience to external and internal shocks (Peacock 2005; Kosgey *et al.* 2008; Ejlertsen *et al.* 2013; de Haan *et al.* 2015).

In addition, sheep and goats can transform vegetation that is inedible or unpalatable for humans, such as grass and foliage growing on non-arable lands, into foods of high quality, bioavailability and nutrient density, such as meat and milk (Otte *et al.* 2012; Smith *et al.* 2013). These products are rich in protein, nutrients and micronutrients that are essential for development and health (Neumann *et al.* 2003; Otte *et al.* 2012; Smith *et al.* 2013; Jodlowski *et al.* 2016). Hence, the presence of small ruminants can have a substantial positive effect on the food and nutrition security in an area (Ayele & Peacock 2003).

In mixed crop-livestock systems, the faeces of small ruminants can be used as manure to improve soil fertility (Peacock 2005; de Haan *et al.* 2015). Sheep and goats are also often used as insurance against crop failure or as a buffer against shocks such as drought or flooding. Crops and livestock are sensitive to different crises; thus, this practice diversifies household risks, improves household resilience, and contributes to food and income stability (de Haan *et al.* 2015). As the SSA region appears to be experiencing extreme climatic events at an increasing rate (Serdeczny *et al.* 2017), this role is likely to become more important in future.

1.2 Infectious diseases as a hindrance to small ruminant production and trade in Sub-Saharan Africa

Given the important roles of small ruminants, it is easy to understand the detrimental effects that infectious diseases in these animals can have (Heffernan 2009; Pradère 2014). Small ruminant diseases can be manifested both as outbreaks of widespread mortalities, or as solitary cases of mild, insidious clinical signs, and everything in between. Maintaining animal health and welfare has been emphasised as a key measure to achieve several of the UN's 17 Sustainable Development Goals (SDGs) (Keeling *et al.* 2019; Olmos Antillón *et al.* 2021), but in spite of this, livestock diseases and their impact on sustainable development remain under-researched. Furthermore, due to severe underreporting of animal diseases across SSA (Grace *et al.* 2012), it is almost impossible to gauge accurately the burden of several animal diseases in the region.

Goats, and to some extent sheep, are generally perceived as healthy and disease resistant compared with many other species (Orskov 2011; Lubungu & Mofya-Mukuka 2012). This perception has been supported by analyses of animal disease data reported to the OIE (The World Bank 2011), however this can also be explained by potential differences in disease reporting between different species. Diseases in small ruminants represent serious problems and can have multiple negative effects on farmer livelihood and wider society. They can lead to poor productivity in various ways, such as limited growth, reduced milk yield, poor reproductive performance and mortalities. In SSA, the high reproductive rate of sheep and goats is countered by high mortality in both adult and young animals (Otte & Chilonda 2002).

1.2.1 Transboundary animal diseases

Many animal diseases have the potential to adversely affect animal health and welfare, as well as human livelihoods. However, this is perhaps particularly prominent in relation to transboundary animal diseases (TADs) (OIE & FAO 2004; FAO 2021). Transboundary animal diseases can be defined as epidemic diseases that are capable of rapid, cross-regional spread and can have serious socio-economic impacts (OIE & FAO 2004; Otte *et al.* 2004; Brown 2011; FAO 2021). They can be disseminated through a variety of means, for example trade, movement of animals and animal products, usage of contaminated biological products, or through migratory animals and wind currents, depending on the pathogen. As TADs are highly contagious and can spread irrespective of national and international borders, regional cooperation is often required to control them (OIE & FAO 2004; Otte *et al.* 2004). They often cause high morbidity and mortality rates in susceptible populations and are therefore a constant threat to the livelihoods of not only farmers, but also other actors within livestock value chains. Furthermore, due to the severe magnitude of their effects, they can also have serious impacts on national economies, due for example to trade bans or disease intervention costs (FAO 2021). With increased globalisation and international trade and travel, there are heightened risks of transboundary diseases suddenly appearing a long way from endemic areas (Otte *et al.* 2004; Domenech *et al.* 2006).

There are several small ruminant diseases that are classified as TADs. Examples include peste des petits ruminants (PPR), contagious caprine pleuropneumonia (CCPP), foot and mouth disease (FMD), and sheeppox (SPP) and goatpox (GTP) (OIE & FAO 2004; Iqbal Yatoo *et al.* 2019). These diseases occur in areas where the majority of the world's poor livestock keepers are found and represent serious threats to livelihoods and wider society. Furthermore, they have a considerable limiting effect on efforts to intensify small ruminant production (Brown 2011; FAO & OIE 2016).

In order to control and limit the negative effects of TADs, detailed knowledge of the epidemiology of these diseases is key. However, there are knowledge gaps concerning the prevalence of these diseases in many parts of the world. In addition, greater knowledge is required of these pathogens' epidemiological characteristics, including the impact of different risk and protective factors on their seroprevalence.

1.2.2 Zoonotic transboundary diseases

A significant number of small ruminant diseases are zoonotic, i.e. they are capable of being transmitted from animals to humans and vice versa. A considerable proportion of the burden caused by these diseases is carried by poor and marginalised communities in, for example, SSA (WHO 2012). Humans are commonly infected when consuming animal products or performing high-risk activities, such as tending to sick or injured animals or slaughtering infected animals (WHO *et al.* 2006). Therefore, certain groups of people are at greater risk of contracting zoonotic diseases, such as livestock farmers, animal traders, slaughterhouse workers, veterinarians and laboratory workers (FAO *et al.* 2006; Angelakis & Raoult 2010).

Several zoonotic diseases are classified as transboundary due to their contagiousness, their severe socio-economic and public health effects, and their ability to spread across regions. Examples include Rift Valley fever (RVF), Crimean-Congo haemorrhagic fever (CCHF), brucellosis and Q-fever (coxiellosis) (OIE & FAO 2004). These diseases can have adverse effects on individual humans and society, for example due to mortality, disability, treatment costs and lost income, in addition to poor production in animals (Perry *et al.* 2002; Rushton *et al.* 2017).

In addition to being transboundary diseases, RVF, CCHF, brucellosis and Q-fever are also frequently classified as neglected zoonotic diseases (NZD). NZD are zoonotic diseases that are often associated with poverty and predominately affect the lives and livelihoods of poor livestock keepers in low- and lower-middle-income countries (King 2011). Due to the considerable impact these diseases can have on human lives and livelihoods, current knowledge regarding e.g. their prevalence in different areas and important transmission pathways needs to be improved.

1.3 Small ruminant production in Zambia and Tanzania

Zambia and Tanzania are two neighbouring countries in south-east Africa (Figure 1). Both countries are classified as least developed countries (LDC) (United Nations 2021) and lower-middle-income countries (The

World Bank 2021). Zambia and Tanzania both struggle with high levels of poverty, especially in rural areas. The majority of the population still live in rural areas, despite high urbanisation rates (The World Bank 2021).

Figure 1: Map displaying the location of Zambia and Tanzania in Africa. Source: Esri, USGS | Esri, © OpenStreetMap contributors, HERE, Garmin, FAO, NOAA, USGS. www. arcgis.com

The main source of income in rural areas in Zambia and Tanzania is crop farming, especially maize farming (Covarrubias *et al.* 2012; Chapoto & Subakanya 2019). However, maize is drought-sensitive, which makes these farmers vulnerable to weather abnormalities and extreme climatic events. In both Zambia (Makondo & Thomas 2020) and Tanzania (McSweeney *et al.* 2006), a trend towards higher average temperatures and reduced amounts of rainfall has been observed, and projections for the future indicate that this development will continue. Studies also indicate that extreme climatic events will become increasingly common (Ngoma *et al.* 2020), making the farming of crops such as maize more and more difficult.

Therefore, livestock is increasingly recognised as a means to improve resilience and promote agricultural diversification (Lubungu & Mofya-Mukuka 2012; Mulenga *et al.* 2019). While the livestock sector's contribution to GDP is low in both Zambia and Tanzania, it constitutes an important source of food, income and livelihood (Covarrubias *et al.* 2012; Tembo *et al.* 2014; Kafle *et al.* 2016). In both countries, small ruminants play important roles. In Zambia, goats are the most commonly kept livestock species after poultry. The goat population is almost 3.6 million, while the number of sheep is around 170 000. More than 99% of the goats and 87% of the sheep are kept by traditional, non-commercial smallholder farmers (Ministry of Fisheries and Livestock 2019). In Tanzania, the goat and sheep populations are around 19 million and 5.6 million respectively, with approximately 99% of the animals kept by traditional smallholders (United Republic of Tanzania 2017). In Zambia and Tanzania, the average herd size of sheep and goats is 7.5 and 7.4 (Ministry of Fisheries and Livestock 2019) and 5.2 and 6.8 respectively (United Republic of Tanzania 2017). However, due to the large variation in herd size in both countries (Covarrubias *et al.* 2012; Ministry of Fisheries and Livestock 2019), with a comparatively small number of farmers keeping large herds and the majority keeping smaller herds, average herd size can be a poor indicator of the number of animals kept by the average farmer. In Zambia for example, most goat and sheep- keeping households keep five goats/sheep or fewer (Ministry of Fisheries and Livestock 2019).

1.3.1 Small ruminant health and disease in Zambia and Tanzania

While small ruminants are generally perceived as comparatively healthy, high endemic disease prevalence and mortality rates are commonly mentioned constraints to the sheep and goat sector in both Zambia and Tanzania (Lovelace *et al.* 1993; MLFD 2010; Covarrubias *et al.* 2012; Namonje-Kapembwa *et al.* 2016; Chipasha *et al.* 2017; Fischer *et al.* 2020a). The health situation is further aggravated by poor access to veterinary services (Kivaria & Kapaga 2002; Chapoto & Subakanya 2019), and in general poor adoption of preventive measures, such as vaccines, anthelmintic drugs and acaricides (Covarrubias *et al.* 2012; Chapoto & Subakanya 2019).

While Zambia and Tanzania are neighbouring countries, there are differences between the two in the small ruminant disease spectrum. In Tanzania, high-impact transboundary diseases, such as PPR and CCPP, are present (Kusiluka *et al.* 2000a; Kusiluka *et al.* 2000b; Torsson *et al.* 2016), while only antibodies to PPR, without linkage to clinical disease, have been detected in Zambia (OIE 2016). However, due to porous borders, the high frequency of cross-border livestock movement and trade, and poor biosecurity, there is a substantial risk of PPR and CCPP being introduced into Zambia from Tanzania (Chazya *et al.* 2014; Karimuribo *et al.* 2014; Chazya *et al.* 2015). This kind of disease introduction could have severe impacts on the small ruminant population and on the sheep and goat farmers in the country, as well as on the Zambian economy. It also poses serious risks to other countries in Southern Africa, e.g. to South Africa and their wool industry.

1.4 Diseases in focus in this thesis

A brief description follows of the pathogens that have been studied as part of this thesis. Some important aspects are summarised in Table 1 below.

Table 1: Short summary of the characteristics of the pathogens studied in this thesis. Footnote references are on the next page

Pathogen	Examples of susceptible species	Zoonosis	Main modes of transmission	Environmental survival	Persistence in animals	Detected in Zambia	Detected in Tanzania
PPRV	Goats, sheep, cattle, certain species of wild ruminants [1,2]	No[1,2]	Direct & indirect contact[3]	Days-weeks[4]	No[4,5]	Antibodies only[6]	Yes[7]
Mccp	Goats, sheep, certain species of wild ruminants[8,9]	No[8,9]	Direct contact, possibly airborne[10,11]	Days-weeks[12]	Possible, not proven[13]	No[14]	Yes[15-17]
FMDV	Cloven-hooved species, incl. cattle, sheep, goats, African buffalos[18]	No (rare reports)[19]	Direct & indirect contact, airborne, insemination, ingestion[20,21]	Days-months[21]	Yes[20-23]	Yes[24-26]	Yes[27,28]
SGPV	Sheep and goats[29]	No[29]	Direct & indirect contact, mechanical vectors (experimentally)[29-31]	Months[32]	No[29]	No	Yes[17]
RVFV	Sheep, goats, cattle, various wildlife species, humans[33]	Yes[33]	Mosquitoes, direct & indirect contact, ingestion[34-36]	Months-years in mosquito eggs[37]	No[37]	Yes[38]	Yes[39]
CCHFV	Cattle, sheep, goats, various wildlife species, humans[40,41]	Yes[40,41]	Ticks, direct & indirect contact, ingestion[40,42]	Years in ticks[43]	Yes (in ticks)[43]	Yes[44]	Yes[45]
Brucella melitensis B. abortus B. suis	Dependent on bacterial species. Goats, sheep, cattle, pigs, humans[46,47]	Yes[46,47]	Direct & indirect contact, ingestion[46,47]	Days-months[47]	Yes[46,47]	Yes[48,49]	Yes[50,51]
Coxiella burnetii	Goats, sheep, cattle, various wildlife species, humans[52]	Yes[52]	Direct & indirect contact, ticks (minor role)[52,53]	Months-years[54,55]	Yes[54,55]	Yes[55]	Yes[56]

[1] Rahman *et al.* (2020)
[2] Rahman *et al.* (2018)
[3] Clemmons *et al.* (2021)
[4] Parida *et al.* (2016)
[5] OIE (2020)
[6] OIE (2016)
[7] Torsson *et al.* (2016)
[8] Ahaduzzaman (2021)
[9] Iqbal Yatoo *et al.* (2019)
[10] Thiaucourt and Bölske (1996)
[11] Lignereux *et al.* (2018)
[12] OIE (2009)
[13] Litamoi *et al.* (1990)
[14] Karimuribo *et al.* (2014)
[15] Kusiluka *et al.* (2000b)
[16] Kusiluka *et al.* (2000a)
[17] Kgotlele *et al.* (2019)
[18] Sinkala *et al.* (2014b)
[19] David and Brown (2001)
[20] Kitching and Hughes (2002)
[21] Paton *et al.* (2018)
[22] Moonen and Schrijver (2000)
[23] Condy *et al.* (1985)
[24] Perry and Hedger (1984)
[25] Hamoonga *et al.* (2014)
[26] Sinkala *et al.* (2014a)
[27] Allepuz *et al.* (2015)
[28] Picado *et al.* (2011)
[29] Tuppurainen *et al.* (2017)
[30] Hamdi *et al.* (2021)
[31] Rao and Bandyopadhyay (2000)
[32] OIE (2013b)
[33] OIE (2019)
[34] Pepin *et al.* (2010)
[35] Ikegami and Makino (2011)
[36] Grossi-Soyster *et al.* (2019)
[37] OIE (2018g)
[38] Dautu *et al.* (2012)
[39] Sindato *et al.* (2014)
[40] Whitehouse (2004)
[41] Fanelli *et al.* (2021)
[42] Fazlalipour *et al.* (2016)
[43] Gargili *et al.* (2017)
[44] Kajihara *et al.* (2021)
[45] Hoogstraal (1979)
[46] Díaz Aparicio (2013)
[47] FAO *et al.* (2006)
[48] Bell *et al.* (1977)
[49] Muma *et al.* (2006)
[50] Shirima and Kunda (2016)
[51] Assenga *et al.* (2015)
[52] Angelakis and Raoult (2010)
[53] ECDC (2010)
[54] Aitken (2007)
[55] OIE (2018a)
[56] Qiu *et al.* (2013)

1.4.1 Peste des petits ruminants (PPR)

Peste des petits ruminants (PPR) is a contagious and frequently fatal disease of sheep and goats, caused by a single-stranded negative-sense RNA-virus, namely peste-des-petits-ruminants virus (PPRV) (species *Small ruminant morbillivirus*). PPRV is a member of the *Paramyxoviridae* family and *Morbillivirus* genus, and is closely related to Rinderpest virus (RPV), canine distemper virus and measles virus (Amarasinghe *et al.* 2019). The disease was first described in Côte d'Ivoire in western Africa in 1942 (Gargadennec & Lalanne 1942), and has since then expanded its range to around 70 countries, mainly across Africa, Asia and the Middle East (OIE & FAO 2015). In 2018, PPR was detected for the first time in the European Union, when an outbreak was reported in Bulgaria (OIE 2018b).

There is only one known serotype of PPRV, however the virus can be subdivided into four different lineages: lineage I predominately present in western Africa, lineage II in western and central Africa and lineage III in eastern Africa. Lineage IV was originally mainly found in Asia and the Middle East, but has since spread and is now widely present across Africa as well (Parida *et al.* 2015b). Small ruminants are the primary hosts of the virus, and while cattle, pigs and camels are susceptible to it, there is currently no indication that they play an important role in the viral epidemiology (Rahman *et al.* 2020). In addition, numerous wildlife species can develop clinical disease following infection, including gazelles, impalas, Nubian ibex and many more. However, their role in the epidemiology is unclear, and there are currently no indications that PPRV circulates in wildlife separately from domestic small ruminants (Rahman *et al.* 2018).

In naïve sheep and goat populations, PPRV can cause morbidity and case fatality rates (CFR) that are as high as 80-100% (Torsson *et al.* 2016). The disease can manifest itself in a per-acute, acute, subacute and subclinical form, and the acute form is the most common (Parida *et al.* 2015a). The per-acute form mainly affects goat kids that are four months or older, when their maternal antibody levels start to wane. The incubation period is short at around two days, after which the animal becomes depressed and pyretic, with body temperatures as high as 40-42 °C. The animal also develops congested mucous membranes, serous oculo-nasal discharge and severe diarrhoea, and will often die within a few days of the onset of clinical signs (Parida *et al.* 2015a).

In the acute form, the incubation period is generally between two and seven days, and is followed by pyrexia of up to 41 °C that can last for up to 10 days. Subsequently, the animal develops serous oculo-nasal discharge and congestion of mucous membranes (Parida *et al.* 2015a). The oculo-nasal discharge can become mucopurulent and occlude the nostrils and cause dyspnea (Parida *et al.* 2015a). Some animals will develop necrotic stomatitis with painful sores on the oral mucous membranes, causing inappetence and halitosis (Roeder & Obi 1999; Torsson *et al.* 2016). After approximately 4-10 days, the animal develops diarrhoea, which is often profuse and blood-stained, and leads to severe, sometimes fatal, states of dehydration (Roeder & Obi 1999; Parida *et al.* 2015a; Torsson *et al.* 2016). Affected animals will sometimes be coughing due to bronchitis and pneumonia (Brown *et al.* 1991) and later become dyspnoeic (Parida *et al.* 2015a). Progressive weight loss is commonly seen and pregnant animals will often abort (Roeder & Obi 1999; Torsson *et al.* 2016). Additionally, the virus causes severe immunosuppression, making the animal more vulnerable to secondary infections. Some animals will recover after 10-15 days post-infection and typically develop lifelong immunity (Roeder & Obi 1999; Parida *et al.* 2015a).

The sub-acute form is mainly seen in endemic areas and only leads to mild clinical signs, such as a slightly elevated body temperature of up to 40 °C. The case fatality rate is generally low, although the impact on production can be substantial (OIE & FAO 2015; Abubakar *et al.* 2016). The subclinical form is mainly seen in large ruminants, such as cattle, where the animal generates a strong antibody response and clears the virus without displaying clinical signs (Parida *et al.* 2015a). According to what is currently known, there is no carrier state or persistent infection (OIE 2020).

PPRV is excreted in body fluids, e.g. tears, coughed secretions, ocular and nasal discharge, and probably also in milk (OIE 2020). The virus is mainly spread through direct contact between infected and naïve animals, but indirect contact with e.g. contaminated pastures and water sources can also play a role (Clemmons *et al.* 2021). However, while PPRV can survive for up to three days in meat, it is rapidly deactivated outside of its host in most other circumstances, which limits the potential for indirect transmission (Parida *et al.* 2016). Also, trade and movement of infected animals are likely to play an important role in the viral epidemiology (Sherman 2011; SADC 2012; Spiegel & Havas 2019; Tounkara *et al.* 2019).

PPR is considered to be one of the most detrimental animal diseases found today, especially in areas where dependence on small ruminants for livelihood is high. The virus is estimated to cause losses of approximately USD 1.5-2 billion annually (OIE & FAO 2015). This occurs in regions that are home to more than 80% of the world's small ruminant population and 330 million of the poorest people on the planet, many of whom depend on sheep and goats for their livelihoods. Therefore, an eradication programme has been launched with the goal to eradicate PPR by 2030. A key measure to achieve viral control is vaccination, and there are several attenuated vaccines available that confer long-term immunity. However, the development of a cost-effective, thermotolerant, DIVA vaccine (i.e. differentiating infected from vaccinated animals) is essential to the successful eradication of the virus (OIE & FAO 2015).

1.4.2 Contagious caprine pleuropneumonia (CCPP)

Contagious caprine pleuropneumonia (CCPP) is caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp). Mccp, previously known as *Mycoplasma* biotype F38, lacks cell wall and belongs to the class *Mollicutes* and the genus *Mycoplasma*, which contains more than 100 different species. The species can be subdivided into four lineages, corresponding to different geographic regions, and Mccp belongs to the *Mycoplasma mycoides* cluster (Iqbal Yatoo *et al.* 2019). CCPP was first described in Algeria in 1873, and since then it has been reported in several countries in Africa, the Middle East and Asia (Asmare *et al.* 2016). CCPP has also been detected in the Thrace region of Turkey, and many suspect that the disease is present in Armenia, Georgia, Greece, and southern Russia, although this has not been confirmed (Ahaduzzaman 2021). While goats are the primary host, Mccp can also cause disease in sheep (Ahaduzzaman 2021) and various wild ruminant species such as the Nubian ibex, Gerenuk and Laristan mouflon, following contact with infected goats (Ahaduzzaman 2021). As Mccp has also been isolated from clinically healthy sheep, the potential reservoir role of this species needs to be further investigated in future studies (Litamoi *et al.* 1990; Iqbal Yatoo *et al.* 2019).

CCPP is highly contagious, with morbidity and CFR of up to 80-100%, and generally higher in exotic breeds and non-endemic areas (Iqbal Yatoo *et al.* 2019). The incubation period is 9-16 days but it can go up to 45 days in rare instances. The disease can manifest itself in per-acute, acute and chronic forms, with the acute form the most common. In the per-acute form, the animal dies within one to three days, generally with minimal clinical signs. In the acute form, the animal generally develops high pyrexia, depression, severe mucopurulent nasal discharge, anorexia, coughing and severe dyspnoea (Smith & Sherman 2009; Ahaduzzaman 2021). Abortion is common in pregnant animals (Thiaucourt & Bölske 1996). Within five days the animal either starts to recover or dies, and at post-mortem inspection, unilateral fibrinous pleuropneumonia is seen with large amounts of mucopurulent to fibrinopurulent exudate (Asmare *et al.* 2016; Iqbal Yatoo *et al.* 2019; Ahaduzzaman 2021). In endemic areas a chronic form of the disease is sometimes seen, with clinical signs often limited to mild nasal discharge and coughing during exercise (Thiaucourt & Bölske 1996; Ahaduzzaman 2021).

The bacterium is spread through inhalation of contaminated droplets at close contact (Thiaucourt & Bölske 1996; Smith & Sherman 2009; Ahaduzzaman 2021). It has been suggested that disease in a herd of sand gazelles resulted from airborne transmission from an unknown source over a distance of at least 50 metres (Lignereux *et al.* 2018). Indirect transmission does not seem to be of particular importance (Thiaucourt & Bölske 1996) and the bacterial survival time outside of the host is short, ranging from up to three days in tropical areas to fourteen days in temperate climates (OIE 2009). There is currently no evidence of a carrier state of the disease (Ahaduzzaman 2021). However, as outbreaks of CCPP have occurred following the introduction of seemingly healthy animals into flocks, this possibility should not be ruled out (Thiaucourt & Bölske 1996).

CCPP is one of the most severe diseases of goats known to man, and the global burden has been estimated to be 507 million USD per annum. The impact on both individual farmers and society is substantial, and Mccp is an impediment to intensification of the small ruminant sector (Iqbal Yatoo *et al.* 2019). If detected early, CCPP can be treated successfully with antibiotics (Smith & Sherman 2009). There is a bacterin vaccine available that has the capacity to prevent clinical disease and resulting economic losses, however it is relatively expensive and frequent revaccination is recommended, making it beyond the reach of most smallholder farmers (Jores *et al.* 2020). There is currently no DIVA vaccine available.

1.4.3 Foot and mouth disease (FMD)

Foot and mouth disease (FMD) is caused by the foot and mouth disease virus (FMDV), a non-enveloped single-stranded RNA virus that is a member of the *Aphtovirus* genus in the *Picornaviridae* family. The virus occurs in seven immunologically distinct serotypes, namely A, O, C, Asia 1, South African Territories (SAT) 1, SAT 2 and SAT 3. All but Asia 1 are present in Africa, and SAT 1-3 are unique to the continent (Vosloo *et al.* 2002). The serotypes do not confer cross-immunity (Kitching 2005) and as FMDV is prone to mutations, new FMDV variants are constantly emerging (Vosloo *et al.* 2002). The virus is enzootic in large areas of Africa, Asia, the Middle East and cause outbreaks in parts of Eastern Europe and South America (Brito *et al.* 2017). The pathogen is thought to circulate in approximately 77% of the global livestock population (OIE 2018e). More than 70 species of wild and domestic cloven-hooved animals, including sheep, goats and cattle, have been found susceptible, although the susceptibility varies considerably between species (Sinkala *et al.* 2014b). Cattle are considered the primary domestic species host, and in wildlife only the African buffalo is known to be able to maintain the virus and play an important role in the viral epidemiology (Sinkala *et al.* 2014b).

While the CFR is low, ranging from around 1-5% in adults to 20% or more in young animals, the virus is extremely contagious and the morbidity rate can approach 100% (OIE 2013a). In small ruminants, FMDV will generally only cause subclinical disease, although clinical cases do occur. The incubation time is around 3-8 days, and the animals may develop pyrexia and vesicles around the coronary band or interdigital space, while oral lesions of the type often seen in cattle are rare (Barnett & Cox 1999). Agalactia in lactating females and lameness are common clinical signs, and lameness can occur both with and without visible foot lesions (Barnett & Cox 1999). Vesicles may also form on the teats and occasionally on the vulva and prepuce, and lactating animals may develop mastitis due to secondary bacterial infections (Kitching & Hughes 2002). The viraemic period, when the infected animal is most contagious, is generally 1-5 days long (Kitching & Hughes 2002). Abortion has been reported and sudden mortalities in lambs and kids resulting from myocarditis is relatively common. In these animals, grey or yellow streaking in the heart, resulting from degeneration and necrosis of the myocardium, is often seen in post-mortem inspection (Kitching & Hughes 2002).

FMDV is excreted in most body fluids, including saliva, faeces, urine, milk and semen, as well as in the breath of an infected animal. Inhalation of viral particles, entry through skin cuts and abrasions as well as ingestion, e.g. of meat and milk, are important viral entry points (Kitching & Hughes 2002; Paton *et al.* 2018). In small ruminants, FMDV is mainly transmitted through direct contact between infected and susceptible animals, and the virus can be excreted several days prior to the development of clinical signs (Kitching & Hughes 2002). Spread by indirect contact with hands, clothing, footwear or vehicles, for example, is also common, and the virus can be disseminated by the wind for up to 60 km over land and 300 km over sea (Hugh-Jones & Wright 1970; Kitching & Hughes 2002; OIE 2021). However, studies in sheep indicate that the distance of aerosol spread of FMDV excreted from this species is considerably smaller (Kitching & Hughes 2002). FMDV can survive for days to months in fodder, organic matter and the environment under suitable cool and moist conditions (Paton *et al.* 2018). Furthermore, FMDV is present in several improperly processed animal products, including meat, milk, offal, bones and hides (Smith & Sherman 2009; Sherman 2011).

The presence of a carrier state can potentially play an important role in the viral epidemiology. Sheep can remain carriers in tonsillar tissues for up to nine weeks and goats for a slightly shorter period (Kitching & Hughes 2002). Cattle generally harbour the virus for six months, but some can remain carriers for up to two years (Hedger 1968; Moonen & Schrijver 2000). Furthermore, African buffalo have been found to retain SAT serotypes for five years or more (Condy *et al.* 1985). While human infection has been described, severe disease is rare, and FMD is generally not considered a public health threat (David & Brown 2001). Humans can, however, harbour the virus for up to 48 hours in their respiratory tract, which has implications for effective disease control in the age of international air travel (OIE 2013a).

Studies in sheep indicate that this species excrete considerably lower amounts of virus than cattle and pigs and therefore are less efficient viral disseminators (Kitching & Hughes 2002). However, the mild and insidious clinical picture in small ruminants enables them to act as a source of the infection for other, more susceptible species, such as cattle (Mansley *et al.* 2003; Smith & Sherman 2009; Sherman 2011). For example, small ruminants have played important roles in disseminating FMDV in previous outbreaks in Turkey and Greece (Sherman 2011), as well as in the FMD outbreak in the UK in 2001 (Mansley *et al.* 2003). Despite the possible role of small ruminants in the epidemiology of FMD, limited attention has been paid to this, and sheep and goats are often omitted from control and vaccination programmes (Sherman 2011).

FMD is one of the most highly regulated livestock diseases in the world, with extensive legislation and regulations in place to limit its spread through the restriction of movement of animals and animal products (Sherman 2011). The annual impact of FMD due to visible production losses and the cost of vaccinations is estimated to be 6.5-21 billion USD in endemic regions, and outbreaks in countries and zones free of FMD are estimated to cause losses of more than 1.5 billion USD per year (Knight-Jones & Rushton 2013). In endemic areas, vaccination is a recommended control measure and DIVA vaccines are available, although immunity is relatively short (Belsham 2020). The vaccines are inactivated to avoid the risk of reversion to virulence, and formulated for the specific viral strain and animal species for which they are intended. Many vaccines confer protection for several strains, but there are currently no vaccines that protects against all viral strains (OIE 2021).

1.4.4 Sheeppox and goatpox (SGP)

Sheeppox (SPP) and goatpox (GTP), here abbreviated as SGP, are ancient viral diseases caused by the sheeppox virus (SPPV) and goatpox virus (GTPV), here named sheeppox and goatpox viruses (SGPV). The viruses are large, enveloped, double-stranded DNA-viruses, classified in the *Capripoxvirus* genus and *Poxviridae* family (Rao & Bandyopadhyay 2000). They are dispersed across the globe and present on multiple continents, including northern and central Africa (predominately north of the Equator), the Middle East and Asia, for example India and China (Tuppurainen *et al.* 2017). Only one serotype is known and while most SGPV strains are host specific, some are able to infect and cause disease in both sheep and goats. There are currently no indications that wildlife plays a role in the viral epidemiology (Tuppurainen *et al.* 2017).

SGPV is highly infectious, with a morbidity rate that can be as high as 70-90%. The CFR is typically around 5-10% in endemic areas, but can reach 100% and is typically higher in exotic breeds, young animals, lactating females and animals reared in intensive systems (OIE 2013b). In addition, the CFR can be high in native breeds if the disease has been absent from a region for some time, or when it occurs together with other pathogens, such as PPRV or FMDV (Rao & Bandyopadhyay 2000; Smith & Sherman 2009; OIE 2013b). The incubation period is generally 4-15 days (Hamdi *et al.* 2021). While the disease is subclinical or mild in some animals, other animals can develop severe, sometimes fatal disease. Early clinical signs include pyrexia, which is followed within a few days by skin macules that later develop into papules, either over the whole body or localised in the groin, axilla and perineum (Rao & Bandyopadhyay 2000; Hamdi *et al.* 2021). In rare cases, the cutaneous lesions can coalesce, a condition that is invariably fatal (OIE 2013b). Furthermore, some animals develop internal lesions, such as in the respiratory and alimentary tract, and also in these animals, mortality is high. Infected animals often stops eating and develops rhinitis, conjunctivitis, mucopurulent ocular and nasal discharge and enlarged regional lymph nodes, as well as lesions on e.g. the external nares, lips, in the mouth, and on the vulva, prepuce, udder and teats (Hamdi *et al.* 2021). Some become dyspnoeic due to pressure on the upper respiratory tract from enlarged retropharyngeal lymph nodes, or from secondary pneumonia (Aitken 2007; Smith & Sherman 2009). In the animals that survive the infection, skin lesions become necrotic after approximately 5-10 days, and the papules start to form skin scabs. The scabs persist for approximately 4-6 weeks and are followed by permanent scarring (OIE 2013b). Recovered animals are generally resistant to re-infection, often also to other capripoxviruses and there is currently no evidence of a carrier state (Tuppurainen *et al.* 2017).

The viruses are mainly transmitted through close contact with infected individuals, through inhalation of aerosolised viral particles and less commonly through contact with mucous membranes or damaged skin (Aitken 2007; Hamdi *et al.* 2021). Transmission can also occur indirectly, e.g. through contact with contaminated bedding, and experimental evidence of transmission with biting fly vectors such as stable flies (*Stomoxys calcitrans*) exist (Tuppurainen *et al.* 2017). After the viral lesions have developed, infected animals excrete SGPV in skin scabs, skin lesions, saliva, ocular and nasal secretions, milk, urine and faeces (Smith & Sherman 2009; Hamdi *et al.* 2021). The animal will stay infectious for approximately one week until the skin lesions have started to become necrotic (Aitken 2007; OIE 2013b). The viruses remain viable for up to three months in wool/hair and skin scabs, and in unclean shaded pens for up to six months (Smith & Sherman 2009; OIE 2013b).

SGPV can cause severe losses through mortalities, poor weight gain, reduced milk production and damage to wool and hides (Garner *et al.* 2000; Smith & Sherman 2009; Limon *et al.* 2020; Hamdi *et al.* 2021). In endemic countries, vaccination is the only effective way to control the spread (Tuppurainen *et al.* 2014). Both live-attenuated and inactivated vaccines have been developed to combat SGPV, however the inactivated vaccines only give short-term immunity, while live attenuated vaccines can give immunity for two years or more and are therefore much more commonly used (Hamdi *et al.* 2021). There are currently no commercial vaccines with a DIVA component (Tuppurainen *et al.* 2017). Most, but not all, vaccines confer some cross-protection for different strains for as well sheeppox and goatpox (OIE 2013b).

1.4.5 Rift Valley fever (RVF)

Rift Valley fever (RVF), caused by the Rift Valley fever virus (RVFV), is a mosquito-borne enveloped negative-sense RNA virus that is a member of the *Phlebovirus* genus in the *Phenuiviridae* family (previously *Bunyaviridae*) (Wright *et al.* 2019). The disease was first described in Kenya's Rift Valley in the 1930s, and has since spread across the African continent, including the island of Madagascar. The disease was first described outside of Africa in 2000, when it was found on the Arabian Peninsula (Sherman 2011). While only one serotype is currently known, there are several different viral strains of variable virulence (OIE 2019).

RVF mainly affects domestic and wild ruminants and humans, while some other species including camels are susceptible but will generally not develop clinical signs (OIE 2019). Sheep, especially lambs, are considered the most severely affected domestic species (Bird *et al.* 2009). The disease typically appears in epizootic outbreaks following heavy rain, sustained flooding or the construction of irrigation schemes and hydrological dams, which in turn is followed by mass-hatching of the mosquito vectors. In general, these high precipitation events occur 5-25 years apart, and the longer the interval, the more animals will be immunologically naïve and hence the outbreak will be more dramatic (Dautu *et al.* 2012).

The incubation period ranges from a few hours to a few days, and is generally at the shorter end of the spectrum for the more susceptible ages and species (Pepin *et al.* 2010). RVF typically manifests itself in epizootic outbreaks characterised by abortions in pregnant females with rates approaching 100%, foetal malformations and mass mortalities in neonatal animals, often due to acute hepatitis. Adult non-pregnant animals are often asymptomatically infected, while some develop a mild febrile illness that is easily overlooked, occasionally combined with ocular and nasal discharge and gastrointestinal signs (Ikegami & Makino 2011). In lambs under two weeks of age, the CFR can be as high as 100%, while in adult animals it typically ranges from 10-20% (Pepin *et al.* 2010). There are currently no indications of a carrier state (OIE 2018g).

Humans will generally develop a mild, self-limiting febrile illness. The incubation period is around 4-6 days, after which symptoms such as malaise, chills, weakness, dizziness, nausea, headache and joint pains can be experienced. Pregnant women may abort (Ikegami & Makino 2011). For most patients, these symptoms will subside, generally within a few days or weeks. However, a small fraction of patients (ranging from 0.5-2%) will develop complications, generally in the form of a haemorrhagic fever, neurological signs resulting from encephalitis, or blindness due to maculopathy or retinopathy (Chevalier *et al.* 2010; WHO 2018). Of these, the haemorrhagic fever has a 50% CFR (WHO 2018), while the neurologic and eye-related complications are rarely fatal but often lead to long-term or permanent damage (Chevalier *et al.* 2010; Ikegami & Makino 2011).

RVFV is transmitted through bites from infected mosquitoes, and the virus has been isolated from multiple mosquito species, although not all of them are necessarily involved in the viral epidemiology. While certain *Aedes* species associated with temporary water bodies are regarded as maintenance vectors, some *Culex* species associated with permanent fresh water are considered epidemic or amplifying vectors (Pepin *et al.* 2010). The virus can be vertically transmitted within some *Aedes* spp. and is known to survive for several years in mosquito eggs in dry conditions that later hatch when conditions become suitable (OIE 2018g). RVFV is thereby maintained in inter-epidemic periods, and the virus is continuously circulating between the vector species and wild and domestic ruminants in some areas (Davies *et al.* 1992; Sumaye *et al.* 2013; Beechler *et al.* 2015; Wensman *et al.* 2015; Mbotha *et al.* 2018; Saasa *et al.* 2018). RVFV can also be transmitted through contact with excretions, tissues and organs from viraemic animals, e.g. at slaughter or through contact with vaginal secretions after abortions (Ikegami & Makino 2011). Humans can also become infected by consuming undercooked meat or raw milk (Grossi-Soyster *et al.* 2019). The infection constitutes an occupational risk for several professional groups, including farmers, veterinarians, abattoir workers and laboratory workers (Bingham & Jansen van Vuren 2020).

There is no specific treatment for RVF, although there are both inactivated and live attenuated vaccines. Inactivated vaccines require several boosters annually, but are considered safe to use on pregnant animals as well, while live vaccines can induce abortions. There is currently no DIVA strategy available for existing RVF vaccines (OIE 2019).

1.4.6 Crimean-Congo haemorrhagic fever (CCHF)

Crimean-Congo haemorrhagic fever (CCHF) is a disease caused by Crimean-Congo haemorrhagic fever virus (CCHFV). CCHFV is a tick-borne, enveloped, single-stranded negative-sense RNA-virus and is a member of the genus *Nairovirus* in the *Nairoviridae* family (Garrison *et al.* 2020). The disease was first described in 1944 in Crimea, Russia, while the virus was first identified in Congo two decades later (Whitehouse 2004). CCHFV can infect a large variety of species, including goats, sheep, cattle, deer, hares, rodents, hedgehogs, camels and humans (Whitehouse 2004; Spengler *et al.* 2016; Fanelli *et al.* 2021). However, CCHFV is only known to cause disease in humans and suckling mice (Fanelli *et al.* 2021). The virus is currently considered endemic in about fifty countries across Asia, Africa, the Middle East and south-east Europe (Nasirian 2020). According to current knowledge, CCHFV only exists as one serotype (OIE 2018f).

While animals are subclinically or inapparently infected, several humans die from CCHFV infection every year. In humans, the disease manifestation generally ranges from asymptomatic infection to mild flu-like symptoms, while some develop a severe, sometimes fatal disease. CCHF is characterised by four distinct phases, namely incubation, pre-haemorrhagic, haemorrhagic and convalescent phase, although the severity and duration of each phase varies greatly among patients (Whitehouse

2004). The incubation period is generally less than a week and is followed by a sudden onset of high fever and symptoms such as chills, headache, dizziness, diarrhoea, vomiting and abdominal pain. This is followed by the haemorrhagic stage, where the patient may develop petechial bleeding, ecchymosis, hematomas and/or bleeding from e.g. the gastrointestinal tract, urinary tract, nose and respiratory tract (Whitehouse 2004). The CFR ranges from 5-80% (Fanelli *et al.* 2021), but averages around 30% (Nasirian 2020; Belobo *et al.* 2021). For the patients who survive the convalescence period follows approximately 2-3 weeks after disease onset. This stage is characterised by e.g. generalised weakness, sweating, headache, dizziness, nausea, loss of memory and occasionally loss of hair. While these problems generally disappear, they may stay with the patient for a year or more (Whitehouse 2004).

CCHFV is transmitted by different tick species, mainly within the *Hyalomma* genus, but also members of the *Rhipicephalus*, *Boophilus*, *Dermacentor* and *Ixodes* genera (Whitehouse 2004). After infection, most animal species will only develop a mild and short-lived viraemia lasting for up to two weeks, during which the animal can transmit the virus (Fanelli *et al.* 2021). As far as is known, there are no indications of an animal species that can carry the virus for a prolonged period of time, however ticks can remain infected throughout their lifetime (Gargili *et al.* 2017). In addition to tick bites, humans can become infected when in contact with infected body fluids and tissues, e.g. when slaughtering a viraemic animal (Rai *et al.* 2008; Mostafavi *et al.* 2017) or when caring for a sick human patient, either at home or in a hospital setting (Whitehouse 2004; Fanelli *et al.* 2021). While CCHFV loses its infectiousness at boiling temperature, and only survives for a short time in meat after slaughter, CCHF cases in humans have been linked to consumption of raw meat (Fazlalipour *et al.* 2016). Interestingly, multiple studies have observed an increased number of CCHF cases in humans in connection with various religious festivals. At these festivals, livestock are brought into towns and cities in large numbers and subsequently slaughtered, often in open areas and by people without training (Rai *et al.* 2008; Nasirian 2020; Butt *et al.* 2021). There are several occupational groups that are at increased risk of infection, including veterinarians, abattoir workers, laboratory workers and hospital workers (Whitehouse 2004). Furthermore, being a livestock farmer is often identified as a risk factor for CCHFV exposure (Monsalve-Arteaga *et al.* 2020).

There is currently no vaccine commercially available for either humans or animals. Therefore, viral control is mainly achieved through information campaigns about how human disease can be prevented, and distributing acaricides to prevent tick bites (OIE 2018f).

1.4.7 Brucellosis

Brucellosis is an ancient zoonotic disease caused by members of the bacterial genus *Brucella* in the *Brucellaceae* family. *Brucella* spp. are gram-negative, facultative intracellular cocco-bacilli that target monocytes and macrophages (FAO *et al.* 2006; Seleem *et al.* 2010). The bacteria are non-motile, non-capsulated and non-spore-forming (Seleem *et al.* 2010). Sheep and goats are typically affected by the most virulent subspecies, i.e. *Brucella (B.) melitensis*. Sporadic infection with *B. abortus* and *B. suis* has also been described, although this is believed to be rare (Rossetti *et al.* 2017). In addition, sheep are susceptible to *B. ovis*, the causative agent of ovine epididymitis, to which goats have been proven susceptible in experimental studies (Burgess *et al.* 1985), but no natural cases have been reported (OIE 2018d). This thesis focuses on brucellosis in sheep, goats and humans caused by *B. melitensis*, *B. abortus* and *B. suis*.

The *Brucellae* tend to be somewhat host-specific, although cross-infections occur, especially for *B. melitensis* and to a lesser extent *B. abortus* and *B. suis*. In addition to small ruminants, *B. melitensis* can also infect e.g. cattle and various wildlife species, often following contact with domestic ruminants (OIE 2018c). *B. abortus* mainly affects cattle and *B. suis* mostly pigs (FAO *et al.* 2006). The world distribution varies with different subspecies, with *B. melitensis* widespread globally and is endemic in many countries in Africa, Asia and the Middle East, as well as parts of South America and the Mediterranean region (Rossetti *et al.* 2017).

Animal brucellosis generally gives rise to a sub-acute or chronic disease. Following infection, a short-lived and transient bacteraemia follows that often will pass without clinical signs, especially in young animals and non-pregnant females (FAO *et al.* 2006). Pregnant animals can develop placentitis, which usually leads to premature birth, the birth of weak offspring, abortions or stillbirths in mid-to-late pregnancy (Rossetti *et al.* 2017). In addition, infected small ruminants may also develop orchitis, epididymitis and arthritis, and brucellosis has been associated with infertility in both sexes (Díaz Aparicio 2013). In general, sexually mature animals are more susceptible, while young animals are more resistant. Especially in sheep, but also in other species, there appear to be differences in breed susceptibility (FAO *et al.* 2006).

Brucellosis in humans is also known as 'undulant fever', 'Mediterranean fever' and 'Malta fever' (FAO *et al.* 2006). The majority of human cases are caused by *B. melitensis*. The clinical manifestation is often vague and the infection can present itself in many atypical forms, which prevents early accurate diagnosis (FAO *et al.* 2006; Dean *et al.* 2012). The incubation time is generally around 2-3 weeks, and for some patients the onset of symptoms is slow and progresses over several weeks (Seleem *et al.* 2010). Clinical signs range from a mild intermittent or remittent febrile illness, which in some patients progresses to a severe, debilitating disease that can be fatal. The patient may also experience general malaise, weakness, inappetence, joint pain, neurologic signs, pneumonia and vomiting, for example. Endocarditis is a relatively common sequelae, and pregnant women may abort (Dean *et al.* 2012). Without treatment, the disease may persist for weeks or months and, in some patients, progress to a chronic form, where clinical signs relapse after a long period of being symptom-free (FAO *et al.* 2006). The CFR is generally low at approximately 0.5%, often caused by endocarditis (WHO 2015).

The bacteria can be excreted in uterine discharges, milk and semen, and animals are generally infected through contact with placenta, foetal fluids and vaginal discharges after parturition or abortion. While subsequent pregnancies are usually carried to term, the infection persists and the animal continues to shed bacteria in the afterbirth and in milk, although generally at lower numbers (Díaz Aparicio 2013). Milk production is often reduced, and permanent infection of the udder is common (FAO *et al.* 2006). Ingestion, inhalation or inoculation of mucosal surfaces or through damaged skin are common infection routes, as well as artificial insemination with infected semen or natural mating with a male shedding bacteria. Humans can become infected when assisting a pregnant female at parturition, removing birth material or when slaughtering an infected animal, for example. Humans can also be infected through consumption of unpasteurised milk and dairy products, and to a lesser extent undercooked meat or offal. Foodborne transmission is generally caused by *B. melitensis*

(FAO *et al.* 2006). In Africa, most human brucellosis cases are caused by direct contact with shedding animals, and foodborne transmission play a more minor role (WHO 2015). Farmers, veterinarians, abattoir workers and laboratory workers are examples of professions that are at increased risk for exposure to the bacteria (FAO *et al.* 2006; OIE 2018c).

The bacterial survival in the environment is short in warm weather, but in colder temperatures in e.g. animal waste and manure, the survival time can be several months (FAO *et al.* 2006; Díaz Aparicio 2013). Of epidemiological importance is the possibility of latent infections, resulting from horizontal infection *in utero* or in the early post-natal period through the consumption of colostrum. While most of these animals clear themselves from the bacteria, some can retain the infection throughout their lives but will generally not seroconvert until after the first abortion or parturition (FAO *et al.* 2006; Díaz Aparicio 2013). Latent infection mainly occurs in cattle, but has also been reported in sheep, and the possibility of it occurring in other species cannot be excluded (FAO *et al.* 2006). These animals pose a serious threat to eradication programmes.

Vaccines are efficient ways to reduce the burden on both animal and human health, especially in endemic areas. Some vaccines are however infectious to humans, can induce abortion in pregnant animals and do not confer an absolute immunity. Unfortunately, the immunological response to this vaccine cannot be differentiated from natural infection (Seleem *et al.* 2010).

1.4.8 Q-fever

Coxiella burnetii, the causative agent of Q-fever, is a ubiquitous acid-fast, gram-negative aerobic obligate intracellular bacterium, and a member of the bacterial family *Coxiellaceae* (Arricau-Bouvery & Rodolakis 2005). The name of the disease is derived from an outbreak of febrile illness in abattoir workers in Brisbane, Australia in 1935 that the physicians were unable to diagnose, and therefore termed it 'Q-fever' for query fever (Angelakis & Raoult 2010). The bacterium is pleiomorphic (coccoid to short rods) and capable of forming endospores that survive for several months in the environment (Arricau-Bouvery & Rodolakis 2005). In nature, the bacterium exists in its virulent Phase 1 stage, also termed 'smooth bacteria'. When cultivated in e.g. cell cultures or embryonated eggs, the bacterium mutates irreversibly to its less virulent or attenuated

Phase II form, also termed ‘rough bacteria’ (Smith & Sherman 2009). *C. burnetii* can be isolated from a large variation of species, including arthropods. However, sheep, goats and cattle are considered the main sources of infection to humans, although cases of infection in dogs, cats, rabbits and birds have also been described. The bacterium is present in most countries across the globe, with the exception of New Zealand (Angelakis & Raoult 2010).

Most infected sheep and goats do not develop clinical signs. However, the infection can cause abortions, usually in late pregnancy, either as a solitary case or in massive outbreaks. Some small ruminants can abort in the subsequent pregnancy (Berri *et al.* 2007). Infection with *C. burnetii* can also lead to stillbirths or birth of weak young (Aitken 2007; Smith & Sherman 2009), and the presence of interstitial pneumonia in young animals in connection with an abortion outbreak has been described (Moore *et al.* 1991). In cattle, associations between *C. burnetii* and metritis and infertility have been described, but this connection has as yet not been made for small ruminants (Aitken 2007; OIE 2018a).

In humans, *C. burnetii* can give rise to a severe debilitating and sometimes fatal disease. The incubation period is generally around 20 days, and in most cases the infection is asymptomatic. Q-fever typically manifests itself in an acute and a chronic form (Roest *et al.* 2011). In the acute form, the patient develops a self-limiting, febrile flu-like illness, sometimes accompanied by atypical pneumonia, hepatitis, myocarditis, pericarditis, skin rashes and neurologic signs caused by meningoencephalitis or peripheral neuropathy. Pregnant women can abort or give birth prematurely (Angelakis & Raoult 2010). In about 2% of patients, the infection will progress into chronic Q-fever (ECDC 2010). This can happen several months or years after the initial infection, and occurs almost exclusively in patients with predisposing conditions, such as immunosuppression, vascular abnormalities and heart valve lesions (Angelakis & Raoult 2010). The most common sequela to chronic Q-fever is endocarditis, but vascular infection, osteomyelitis, pneumonia, hepatitis or chronic fatigue syndrome are also common (Angelakis & Raoult 2010). The case-fatality rate is 1-2% for acute and 5-50% for chronic Q-fever (ECDC 2010).

Infected sheep and goats shed the bacterium in large numbers in placenta, aborted foetuses, faeces, milk and vaginal secretions following both abortion and normal parturition. The vaginal shedding typically peaks at the time of abortion or parturition, but some animals may continue to shed for multiple months. For some females, the vaginal shedding can resume in subsequent pregnancies (Aitken 2007). Additionally, the animal may shed *C. burnetii* intermittently in milk for up to three months after parturition (Berri *et al.* 2005). The bacterium is transmitted both directly and indirectly. New hosts are mainly infected through inhalation, and the organism can be spread with the wind for up to five kilometres (ECDC 2010). The bacterial spores survive for up to four months in dust, 12-16 months in wool and 18 months in tick faeces (Aitken 2007). Indirect transmission can occur through contact with e.g. contaminated hay, manure and wool or grazing on contaminated pastures. While *C. burnetii* persists in around forty different tick species, ticks are not believed to play a significant role in the epidemiology other than for wildlife species. Humans can get infected when assisting animals at parturition or at slaughter, for example. Ingestion, particularly of unpasteurised milk, is a possibility, but the importance of this transmission pathway is currently unclear (Angelakis & Raoult 2010). The bacterium is a clear occupational hazard for multiple professions, including farmers, laboratory personnel, abattoir workers and veterinarians (Angelakis & Raoult 2010; OIE 2018a).

Both animals and humans can be treated with tetracycline. In animals, treatment does not prevent bacterial shedding, but it may reduce abortion rates during an outbreak, and in some countries, treatment of all pregnant small ruminants in an affected herd is recommended (Aitken 2007; Smith & Sherman 2009). The acute form in humans generally resolves quickly with or without antibiotic treatment. However, the chronic form requires prolonged treatment for up to two years, and can result in death if left untreated (Angelakis & Raoult 2010). There are inactivated vaccines commercially available, but while they confer good protection, repeated annual vaccination is recommended, especially of young animals. There is also a human vaccine, but it is not widely available (OIE 2018a). Unfortunately, there is currently no DIVA vaccine available (Rousset *et al.* 2021).

1.5 Livestock trade and movement and the potential risks for disease dissemination

As a response to population growth, urbanisation and improved livelihoods, the demand for animal-derived products around the world is increasing, especially in developing countries (Delgado *et al.* 1999). While this demand is slowing down in many parts of the world, it remains high in Africa, especially in urban areas (Latino *et al.* 2020). With the continuously high population growth (Suzuki 2019), urbanisation rate (Saghir & Santoro 2018) and improved living standards in SSA, the annual meat demand is projected to have doubled in the region by 2050 (Robinson *et al.* 2011). This trend has also been seen in the small ruminant sector, as the demand for mutton meat is projected to increase by 137% in the next few decades, with the greatest increase in SSA and South Asia (FAO & OIE 2016). This represents a golden opportunity for the rural poor to venture into the livestock value chain, e.g. as farmers, traders or retailers, to reap the benefits of this growing demand. Therefore, taking measures to increase the trade of animals and animal products has been emphasised as an important measure to alleviate poverty (Delgado *et al.* 1999; ILRI 2002; Scoones & Wolmer 2006).

1.5.1 Trade and disease dissemination

However, while the increased demand for small ruminant meat represents an opportunity for farmers to move out of poverty, it can also contribute to the dissemination of infectious diseases. By moving animals or animal products from one area to another, diseases can be introduced into new regions. If a livestock disease is introduced into an area where the animal population is naïve, the potential impacts can be severe for the individual animals and farmers as well as for society (Sherman 2011). There are numerous examples of infectious diseases that have been widely disseminated through animal movement and trade, including PPR (Kivaria *et al.* 2013), RVF (Sherman 2011), SGP (Limon *et al.* 2020), and Q-fever (Sanford *et al.* 1994). This clearly illustrates the immediate necessity to study the impact of trade on disease epidemiology, and improve understanding of trade patterns and other important mechanisms that can contribute to increased or decreased disease dissemination linked to trade.

In SSA, the trade with small ruminants mainly occurs locally, with the majority taking place in the informal sector (Scoones & Wolmer 2006;

Perry & Grace 2009; Sherman 2011; de Haan *et al.* 2015; FAO & OIE 2016). Informal trade can be defined as trade that is unrecognised or unrecorded by the government, usually without adhering to different legislations on e.g. biosecurity and sanitation. Therefore, it is likely that the risk of disease spread is particularly large within this channel (Little 2005; Grace & Little 2020), and it can therefore be argued that this sector should be targeted by research and intervention programmes.

1.5.2 Live animal markets and the dissemination of disease

Considerable amounts of animal trade are conducted at informal live animal markets. These markets are important sources of income for various value chain members, including farmers, traders, abattoir workers and various retailers (Lysholm *et al.* 2020; Naguib *et al.* 2021; WHO 2021), and they constitute important sources of animal-derived food in many parts of the world, including SSA (Naguib *et al.* 2021). As many in the SSA region lack access to cold storage, they prefer to buy live animals or fresh products at live animal markets for immediate slaughter and or consumption (Naguib *et al.* 2021; WHO 2021). While these markets are frequented by people in all income classes, their comparatively low prices make them particularly important to the urban poor (Hichaambwa 2012).

However, live animal markets, especially informal animal markets, can also play an important role in the emergence and dissemination of infectious pathogens. By bringing different species into close contact, often under stressful and poor hygiene conditions, these markets create an interface where pathogens can emerge and spread, both within and between species (Guan *et al.* 2003; Lysholm *et al.* 2020; Naguib *et al.* 2021; WHO 2021). There are several examples of how animal diseases have been disseminated at or in connection to animal markets, e.g. FMD (Mansley *et al.* 2003; Ortiz-Pelaez *et al.* 2006; Robinson & Christley 2007) and sleeping sickness (Fèvre *et al.* 2001), and at goat markets in Nepal, PPRV RNA and FMDV RNA have been detected in environmental samples (Colenutt *et al.* 2021). In addition, outbreaks of zoonotic disease in humans have been linked to animal markets on multiple occasions, such as the severe Q-fever outbreak in Germany in 2003 (Porten *et al.* 2006). Furthermore, animal markets have been associated with the emergence of pandemic diseases, such as avian influenza (Wan *et al.* 2011; Zhang *et al.* 2014; Zhou *et al.* 2015; Zhou *et al.* 2016), SARS (CDC 2003; Guan *et al.*

2003) and, most recently, Covid-19 (Huang *et al.* 2020; Ren *et al.* 2020; Zhang *et al.* 2020; WHO 2021). This clearly illustrates the pressing need to address current knowledge gaps concerning the risks of disease dissemination at informal live animal markets. This includes risks for zoonotic disease spread, including those related to slaughter or consumption of potentially contaminated food.

1.5.3 The animal trader

The animal trader holds a key position in the trade process and can act to both contain and spread disease (Mubamba *et al.* 2018). It is therefore highly relevant to understand how this actor understands and act on animal disease. Previous research has mainly been in the form of quantitative questionnaire studies, and has often focused on determining the traders' factual knowledge levels (Neupane *et al.* 2012; Yu *et al.* 2013; Leslie *et al.* 2016; Elelu 2017). A common conclusion is that the traders have insufficient knowledge regarding aspects such as how animal diseases are transmitted. These studies often assume a direct causal link between individual knowledge and behaviour. Therefore, they often suggest that traders need to be educated about animal disease in order to reduce their tendencies to engage in disease transmitting behaviours.

However, in a small number of qualitative studies investigating pig traders' perceptions and practices related to African swine fever (ASF), the authors' conclude that traders are generally knowledgeable about important aspects such as disease transmission routes. The studies also indicate that traders are aware of their potential role in disease dissemination, but nevertheless frequently engage in risky activities (Dione *et al.* 2016; Chenais *et al.* 2017; Lichoti *et al.* 2017). This implies that the traders' risky behaviours are not solely caused by poor knowledge. More research is needed to understand why traders engage in disease-transmitting activities and to map the structural limitations that prevent them from taking fewer risks.

1.5.4 The impact of animal movement and trade close to international borders and larger roads – the example of FMD in Zambia and Tanzania

In SSA, regional cross-border trade and movement of animals is common, both in formal and informal networks (Di Nardo *et al.* 2011; de

Haan *et al.* 2015). As international borders typically are crossed at specific crossing points, the frequency of animal movement is often increased there (Di Nardo *et al.* 2011), which in turn can lead to increased pathogen prevalence. In spatiotemporal studies in Zambia and Tanzania, clustering of FMD outbreaks in cattle has been observed close to several international borders (Perry & Hedger 1984; Picado *et al.* 2011; Hamoonga *et al.* 2014; Sinkala *et al.* 2014a; Allepuz *et al.* 2015). In Tanzania, FMD outbreaks mainly occurred along the borders with Kenya, Uganda, Rwanda, Burundi, the DRC and Zambia (Picado *et al.* 2011; Allepuz *et al.* 2015). In Zambia, the outbreaks were clustered along the borders with Zimbabwe, Botswana, Namibia and Tanzania (Perry & Hedger 1984; Hamoonga *et al.* 2014; Sinkala *et al.* 2014a). Furthermore, clustering has been observed close to main roads in both Tanzania (Allepuz *et al.* 2015) and Zambia (Hamoonga *et al.* 2014). This includes the Tanzania-Zambia highway, or Tan-Zam highway, which is an important road that connects the port in Dar Es Salaam, Tanzania, with the Zambian capital Lusaka and beyond (Allepuz *et al.* 2015). The findings of these studies indicate that both international trade and the national movement of cattle, plays an important role in the epidemiology of FMDV in the region. Whether this finding also applies to other areas, species and pathogens has yet to be determined.

2. Aims of the thesis

The overall aim of this thesis was to improve understanding of the circulation of selected transboundary pathogens in sheep and goats on farms and in markets in Zambia and in the Tanzania-Zambia border region. Its more specific aims were to:

- investigate the seroprevalence of PPRV, FMDV, SGPV, RVFV and *Brucella* spp. in sheep and goats in the Tanzania-Zambia border region (Paper I)
- investigate the seroprevalence of Mccp, FMDV, SGPV, RVFV and *Brucella* spp. in goats in four Zambian border districts and three inland districts (Paper II)
- assess the impact on seroprevalence of selected risk factors, focusing on trade routines and proximity to international borders (Papers I & II)
- explore the perceptions and practices of small ruminant traders on sheep and goat health and disease, and the influence these have on the risk of disease dissemination through trade (Paper III)
- investigate the seropositivity rate of the zoonotic pathogens *Brucella* spp., *Coxiella burnetii* and RVFV in sheep and goats at two urban informal small livestock markets in Zambia (Paper IV).
- document slaughter routines, procedures and hygiene at an informal slaughterhouse to understand the risks for occupational exposure to zoonotic disease at slaughter and for contamination of meat and viscera (Paper IV)

Figure 2: The author collecting blood samples at a small livestock market in Zambia. Photograph: Christabel Chimba

3. Comments on materials and methods

This section contains an overview of, and comments on, the materials and methods used in Papers I-IV. For more detailed information regarding the methodology, please refer to the individual papers.

3.1 General study aspects

This thesis is based on data collected during three field trips to Zambia and Tanzania and has resulted in Papers I-IV. A schematic overview of the field work on which the thesis is based is presented in Table 2 below.

Table 2: Schematic overview of the field work data on which this thesis is based, including the time, location and focus of each trip, and the resulting scientific study

Data collection period	Location	Activity	Paper
April-May 2018	Lusaka small livestock market	Semi-structured interviews - traders and other value chain actors Observations - market activities	III
		Semi-structured interviews - slaughterhouse workers and other value chain actors Focus group discussions - slaughterhouse workers Observations - Lusaka market slaughterhouse	IV
Sept-Oct 2018	Lusaka and Kasumbalesa small livestock markets	Semi-structured interviews - slaughterhouse workers and other value chain actors Observations - Lusaka market slaughterhouse Serum samples from sheep and goats	IV
	Tanzania-Zambia border region	Serum samples from sheep and goats	I
Sept-Nov 2019	Four Zambian districts with an international border, three Zambian inland districts	Serum samples from sheep and goats	II

3.2 Selection of pathogens (Papers I, II and IV)

This thesis aimed to explore the circulation of transboundary diseases in farms and markets in Zambia and in the Tanzania-Zambia border region. In total, eight different pathogens were included in the thesis. These pathogens were selected based on their importance in the region and their impact on the individual and on society. Furthermore, pathogens with as wide a variety of epidemiologic characteristics as possible were chosen, and preference was given to those where livestock movement and trade played a substantial role in the dissemination. Among the zoonotic pathogens, preference was given to neglected zoonotic pathogens that are transmissible to humans during the slaughter of infected animals or through consumption of undercooked meat.

3.2.1 Peste des petits ruminants virus (PPRV) (Paper I)

PPRV was chosen because it is often described as the most important animal pathogen in areas where the dependence on small ruminants for livelihoods is high. PPRV was also chosen because while PPRV is endemic in Tanzania (Torsson *et al.* 2016) and PPRV nucleic acid has been identified on multiple occasions in samples from different parts of the country (Muse *et al.* 2012; Kivaria *et al.* 2013; Kgotlele *et al.* 2014; Mahapatra *et al.* 2015; Misinzo *et al.* 2015; Kgotlele *et al.* 2019; Jones *et al.* 2020), only antibodies without linkage to clinical disease have been found in Zambia (OIE 2016). However, due to porous borders, poor biosecurity and the high frequency of cross-border animal movement and trade, the risk of PPRV being introduced to Zambia from Tanzania is high (Chazya *et al.* 2014; Chazya *et al.* 2015). PPRV has also been detected in neighbouring DRC (FAO 2012; Birindwa *et al.* 2017; Tshilenge *et al.* 2019) and Angola (OIE 2013c). The OIE and FAO aims to eradicate PPRV by 2030 and to achieve this goal, knowledge of the seroprevalence and important epidemiological characteristics in different areas is key (OIE & FAO 2015). This thesis aims to contribute with information on these aspects in the Tanzania-Zambia border region.

3.2.2 *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) (Paper II)

Mccp was chosen because it is considered one of the world's most detrimental diseases affecting goats (Asmare *et al.* 2016; Ahaduzzaman 2021). Mccp is present in Tanzania (Kusiluka *et al.* 2000a; Kusiluka *et al.* 2000b; Kgotlele *et al.* 2019), but has yet to be detected in Zambia. However, due to the high frequencies of cross-border animal movement and trade, as well as poor biosecurity in the region, there is a clear risk of Mccp being introduced into Zambia from Tanzania (Karimuribo *et al.* 2014). This would have detrimental effects on the small ruminant population in the region and on the people who depend on them for their livelihoods.

3.2.3 Foot and mouth disease virus (FMDV) (Papers I and II)

FMDV was selected as it is often put forward as the most important transboundary animal disease on the planet (OIE & FAO 2004) due to its high morbidity rate and severe impact on animal industries and the economy through production losses and trade impairments (Sherman 2011). The virus is endemic in both Zambia (Hamoonga *et al.* 2014; Sinkala *et al.* 2014a) and Tanzania (Picado *et al.* 2011; Allepuz *et al.* 2015), although in Zambia, research has thus far been focused on cattle. As small ruminants often are subclinically infected and frequently are left out from e.g. vaccination programs (Mansley *et al.* 2003; Sherman 2011), they can act as a source of infection for more susceptible species such as cattle and contribute to the dissemination of FMDV over large distances. Therefore, improved understanding of the viral epidemiology in these species is important to control the virus.

3.2.4 Sheeppox and goatpox virus (SGPV) (Paper I)

SGPV was chosen because of its severe effects on small ruminant populations and smallholder livelihoods (Garner *et al.* 2000; Limon *et al.* 2020). The viruses are major constraints to the intensification of small ruminant production (Hamdi *et al.* 2021) which many argue is a necessity to meet the projected increased demand for mutton meat in SSA (FAO & OIE 2016). The presence of SGPV has been confirmed in Tanzania (Kgotlele *et al.* 2019) as well as in neighbouring DRC (Birindwa *et al.* 2017), but has yet to be detected in Zambia. Seroprevalence studies in SSA are few, and more information is needed on the presence and circulation of SGPV in the region.

3.2.5 Rift Valley fever virus (RVFV) (Papers I, II and IV)

RVF is a transboundary, mosquito-borne, neglected zoonotic disease that has a considerable impact on animal populations, individual farmers and society at large. The disease is endemic in both Zambia and Tanzania, although several years have passed since the last outbreaks (Dautu *et al.* 2012; Sindato *et al.* 2014; Ahmed *et al.* 2018). Slaughterhouse workers are at increased risk of exposure to RVFV (Ikegami & Makino 2011) and people can become infected through the consumption of undercooked meat (Grossi-Soyster *et al.* 2019). To reduce the disease burden in sheep, goats and humans in Zambia and Tanzania, improved knowledge is needed on the circulation of RVFV in the small ruminant populations. Also, the presence of the virus at livestock markets needs to be investigated because of the high risk of human exposure at these locations.

3.2.6 Crimean-Congo haemorrhagic fever virus (CCHFV) (Paper II)

CCHF is a transboundary neglected zoonotic disease that, despite the inapparent infection in most animal species, can lead to a severe, sometimes fatal disease in humans (Whitehouse 2004). Therefore, while the impact on livestock production is negligible, CCHFV can have a substantial public health impact. Serological evidence of CCHFV circulation has been detected in both Zambia (Kajihara *et al.* 2021) and Tanzania (Hoogstraal 1979), as well as viral genetic material in ticks in Zambia (Kajihara *et al.* 2021). Small ruminants and cattle play a crucial role in the epidemiology of CCHF in humans (Spengler *et al.* 2016) and serological screening in ruminants can help identify areas in which CCHFV is circulating. These areas can then be targeted by various control measures, such as information campaigns and tick preventative measures (OIE 2018f).

3.2.7 *Brucella* spp. (Papers I, II and IV)

Brucellosis is a neglected zoonotic disease that causes a severe burden across the globe, particularly in developing countries (Grace *et al.* 2012). The disease is present in both Zambia (Bell *et al.* 1977) and Tanzania (Mellau *et al.* 2009; Assenga *et al.* 2015; Shirima & Kunda 2016), although research in Zambia has mainly focused on cattle (Muma *et al.* 2006; Muma *et al.* 2007a; Muma *et al.* 2007b; Muma *et al.* 2008; Muma *et al.* 2013; Mfune *et al.* 2021). As most human cases are caused by *B. melitensis* which mainly occurs in small ruminants, more research on brucellosis in these species is warranted. Humans can become infected both through contact with body fluids at slaughter and when consuming undercooked meat (WHO 2015). Therefore, investigating the presence of the bacteria at small livestock markets and risks for disease spread in these locations is desirable.

3.2.8 *Coxiella burnetii* (Paper IV)

C. burnetii, the causative agent of Q-fever, is a neglected zoonotic pathogen that has a substantial impact on animal populations, individual farmers and wider society. The burden is especially large in low- and lower-middle-income countries (Grace *et al.* 2012). Small ruminants are important sources of human infection and cases of human disease have been linked both to visiting a live animal market (Porten *et al.* 2006) and to dwelling in the vicinity of a slaughterhouse (Carrieri *et al.* 2002). Therefore, investigating if *C. burnetii* is circulating in source populations for markets and slaughterhouses is highly relevant.

3.3 Study areas (Papers I and II)

Paper I is based on research conducted in the Tanzania-Zambia border region. The aim was to assess the impact of certain risk factors on the seroprevalence of selected transboundary pathogens in sheep and goats, focusing on trade routines and proximity to an international border, to a town and to the Tan-Zam highway. This region was purposively selected as PPRV is considered endemic in Tanzania (Karimuribo *et al.* 2011; Kivaria *et al.* 2013; Swai *et al.* 2013; Torsson *et al.* 2017; Kgotlele *et al.* 2019), while only antibodies with no connection to clinical disease have been found in Zambia (OIE 2016). Furthermore, the region has been identified as a hotspot for FMD outbreaks in cattle on both sides of the border (Perry & Hedger 1984; Picado *et al.* 2011; Hamoonga *et al.* 2014; Sinkala *et al.* 2014a; Allepuz *et al.* 2015). Clusters of FMD outbreaks have also been found along the Tan-Zam highway and Tazara railway (Hamoonga *et al.* 2014; Allepuz *et al.* 2015). These are two important trade and communication infrastructures that connect the Zambian capital of Lusaka with the port to the Indian Ocean in Dar Es Salaam, Tanzania. Two districts were purposively selected on both sides of the border in collaboration with local partners. The choice was based on proximity to the Tanzania-Zambia border, the presence of small ruminants, and the local district veterinary officer giving permission for the research to be conducted. Furthermore, one district in Zambia and two districts in Tanzania were purposively chosen since the Tan-Zam highway and Tazara railway run through them (Figure 3).

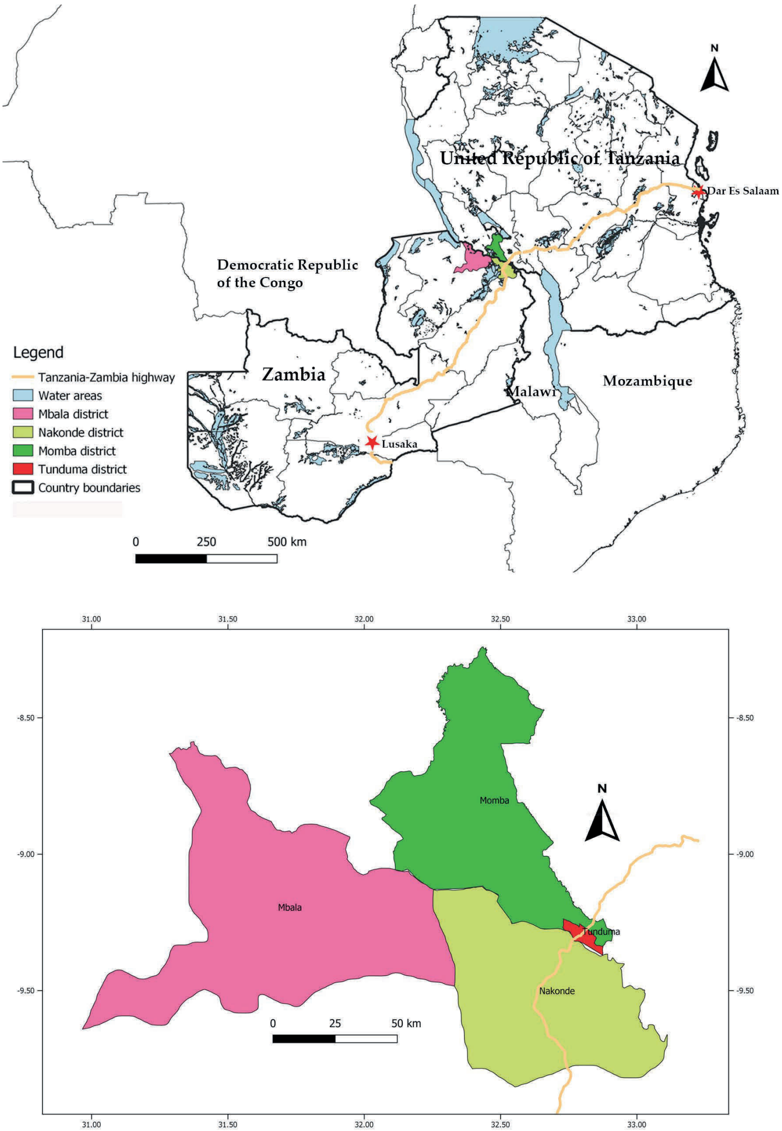

Figure 3: Location of the visited districts in the Tanzania-Zambia border region, and of the Tanzania-Zambia highway. Map created in QGIS version 3.4.4 software (https://qgis.org) and kindly provided by Dr Jean Hakizimana.

The aim of the study in Paper II was to continue to investigate the impact of certain risk factors, focusing on border proximity and trade, on the seroprevalence of selected transboundary pathogens of small ruminants. Seven districts were purposively selected in collaboration with local partners: four were adjacent to one or more international borders, and three were inland districts (Figure 4). The choice of districts was based on their location in the country (i.e. border or non-border district), goat density (Namonje-Kapembwa *et al.* 2016) and the local district veterinary officer giving permission for the research to be conducted. Districts bordering Malawi, Mozambique and Zimbabwe were included as cross-border trade of sheep and goats between these countries and Zambia was common according to local contacts. One district bordering Angola and one bordering the Democratic Republic of Congo (DRC) were also selected as PPRV has been detected in both countries (FAO 2012; OIE 2013c; Birindwa *et al.* 2017; Tshilenge *et al.* 2019). Furthermore, the district bordering the DRC was chosen based on the presence in the district of Kasumbalesa market, i.e. a large market for small livestock where a lot of international trade between Zambia and the DRC takes place. The inland districts were chosen based on their accessibility from the capital Lusaka.

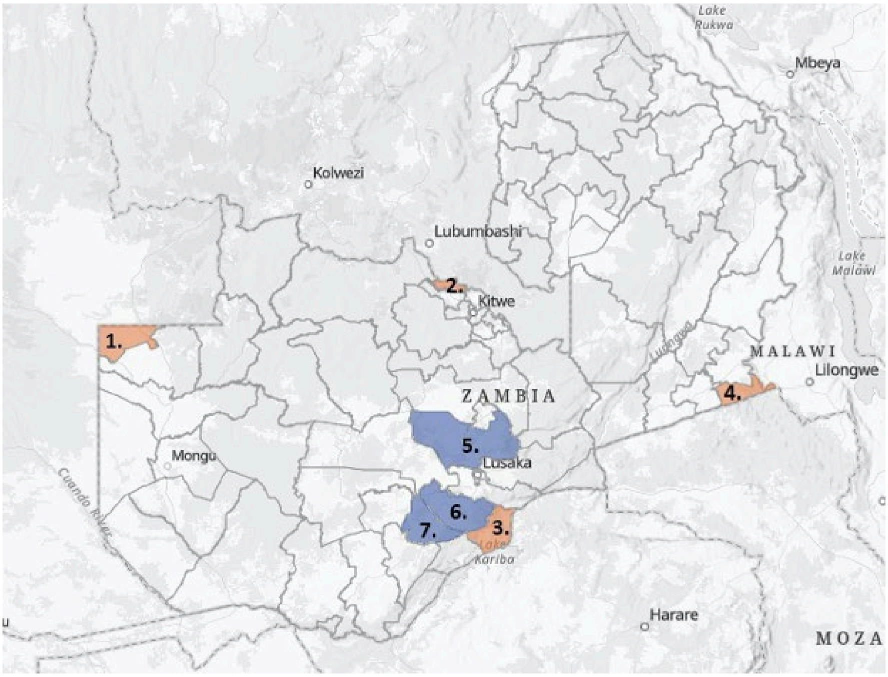

Figure 4: Map of Zambia showing the locations of the visited districts: 1=Chavuma, 2=Chililalombwe, 3=Siavonga, 4=Vubwi, 5=Chibombo, 6=Mazabuka, 7=Monze. Map kindly provided by Emma Lysholm. Source: Esri, USGS | Esri, © OpenStreetMap contributors, HERE, Garmin, FAO, NOAA, USGS. www.arcgis.com

3.4 Study design and the challenges of collecting animal samples in developing countries (Papers I and II)

The studies in Papers I and II were conducted as cross-sectional studies and aimed to investigate the impact of certain risk factors, such as trade and border proximity, on the seroprevalence of selected transboundary pathogens. In Paper I, both sheep and goats were targeted, while only goats were included in Paper II as the number of sheep was low. Both studies were conducted in two strata, namely Tanzania vs. Zambia in Paper I, and border districts vs. non-border districts in Paper II. The individual animal was the primary sampling unit. The sample size per stratum was calculated using the Epitools online calculator 'Sample size to estimate a true prevalence with an imperfect test', which can be found on the webpage www.epitools.ausvet.com.au (Ausvet, Australia). The calculator is based on the instructions and formulae in Humphry *et al.* (2004). In the calculation, an infinite population size was assumed, and a 95% confidence interval (CI), 5% margin of error and 50% assumed true prevalence were used, along with the sensitivity and specificity values from the ELISA with the lowest values, in order to generate the largest required sample size.

This sample size calculation is based on a simple random sample and did hence not take clustering in e.g. herds, villages and districts into account. Clustering was disregarded as the small ruminant population structures in both Zambia and Tanzania are largely unknown. The design effect was not estimated due to the lack of information on e.g. the composition of the sheep and goat populations and on disease prevalence in the two countries (especially Zambia) and due to the inclusion of multiple pathogens in the studies. To compensate for this, the sample size calculation was made using a 50% assumed true prevalence and the sensitivity and specificity values from the ELISA with the lowest values, to generate a sample size estimation that was as large as possible. Under ideal circumstances, however, the studies on which Papers I and II are based would have been designed as multi-staged cluster sampling (Thrusfield 2005), preferably with access to secondary data of the number of farms and animals present, and following pilot studies investigating disease prevalence in the different areas.

3.4.1 Selection of villages, households, and animals (Papers I and II)

As most of the included pathogens in both studies are contagious and have high morbidity rates, the hypothesis was that if a herd has been exposed to a pathogen, a large proportion of the animals will be seropositive. Furthermore, as small ruminants are often grazed communally and meet at common water points, they are in frequent contact with sheep and goats from other herds within the village, and can to a large extent be expected to share pathogen burden. Therefore, the aim was to include as many villages and herds/households keeping small ruminants as possible, while at the same time maintaining a feasible sample scheme.

In both Papers I and II, village lists were collected from local veterinary personnel and a random selection performed from these lists using the 'Randomize tool' in Microsoft Excel (Washington, USA). These lists were later revised with the local district veterinary staff, and villages that were inaccessible or did not contain small ruminants were replaced. In Paper I, the goal was to sample four herds per village. In Paper II, four herds per village were sampled in the districts situated along an international border, while three herds per village were targeted in the inland districts. If few small ruminant herds were present in a village, all the herds that gave consent were included and the number was complemented with herds in the next village on the list or with herds from nearby villages. The herd selection was performed using snowball sampling methodology, which is a very efficient method to identify and include members of a target population about which limited information is available and that may be difficult to locate (Kendall *et al.* 2008; Hennink *et al.* 2020), such as owners of small ruminants in Zambia and Tanzania. In brief, after identifying the first small ruminant farmer, this farmer then directed the research team to the next farmer, and so on. Hence, a main drawback is that the selection is dependent upon previous participants, which constitutes a risk for bias. For example, neighbours and farmers with a wide social network are more likely to be included, whereas minority groups, or even majorities that do not happen to be within the same social network as the first farmer, are at risk of being excluded (Hennink *et al.* 2020). In Paper I, attempts were made to control for this bias and diversify the farmers being included in the research by stratifying the selection based on herd size. Hence, the farmers were asked to direct the research team to someone who fulfilled one of the following conditions:

- kept fewer than five sheep and/or goats
- kept 5-15 sheep and/or goats
- kept more than 15 sheep and/or goats.

The goal was to include at least one herd from each herd size group per village. If all three of the above-listed groups had been included with the first three herds, no conditions were given for the last herd, meaning that the farmer could direct the research team to any small ruminant herd, regardless of its size. If it was not possible to find representatives from all groups, another herd was included irrespective of the group to which it belonged. However, this stratification was not possible in many villages, especially in Zambia, as the number of small ruminant herds was small. Consequently, this stratification strategy was not used in Paper II. Instead, the farmer was just asked to direct the research team to a goat herd, irrespective of its size.

The stratification groups were formulated in collaboration with local partners and based on the average herd sizes for sheep and goats in Zambia (7.4 and 7.5, respectively) (Ministry of Fisheries and Livestock 2019) and Tanzania (6.8 and 5.2, respectively) (United Republic of Tanzania 2017). However, the variation in herd size is considerable in both countries (Covarrubias *et al.* 2012; Ministry of Fisheries and Livestock 2019) and therefore average herd size is probably a poor indicator of the number of animals kept by the average Zambian or Tanzanian farmer. Therefore, if this study were to be done again, this stratification would be omitted from the study design.

The choice of which individual sheep or goats to sample was generally non-random. By the time the research team arrived in the villages to collect the samples, on several occasions the sheep and goats had already been let out to graze. In these instances, the first animals caught were generally sampled due to time constraint. As animals that are suffering or recovering from disease are often weak, this may have influenced the serological results. In instances where the animals were tethered or enclosed, an attempt was made to randomise the selection by sampling every other animal or every third animal for example. However, in many instances, the farmers wanted to choose which animals were sampled. It is possible that the farmers chose to propose the healthiest animals due to a desire to present themselves as good farmers. However, farmers could also choose to present the sickest animals out of a desire to find out what was causing the animal's illness. The process when the farmers chose which animal should be sampled generally appeared random, and it is not possible to estimate whether one of the two options was more common than the other.

3.4.2 Serum sample collection (Papers I and II)

For each sampled individual, serum samples were collected from the jugular vein, along with information on age, sex, origin and disease signs, both on the day of sample collection and within the last year. The serum was stored standing in cool boxes during the day, and at the end of each day the separated serum was transferred to cryotubes that were subsequently placed in a -20 °C freezer. The research team rarely had access to centrifuges to spin the samples prior to separating the serum.

Electric load shedding and unforeseen power cuts happened on an almost daily basis when the samples included in Paper II were collected, and frequently also during the data collection for Paper I. As access to generators is limited in rural Zambia and Tanzania, the freezers stopped working at these times, which caused problems in terms of sample storage. Fortunately, the electric power cuts never lasted more than a few hours. As soon as possible, the samples were transferred to a -80 °C freezer for long-term storage.

3.4.3 Questionnaire interview (Papers I and II)

A questionnaire was used to collect information on trade routines, management practices, contact with domestic and wild ruminants, and herd disease history. The questionnaire was written in English and translated into the local language by an enumerator. In Tanzania, the questionnaire interviews were performed in Kiswahili. In Zambia, the local language differed in different regions. In Paper I, Namwanga was used in Nakonde district and Mambwe in Momba district. In Paper II, Tonga was spoken in Monze, Mazabuka and Siavonga districts, Lenya or Nyanja in Chibombo district, Bemba in Chililalombwe district, Chewa in Vubwi district, and Luvale in Chavuma district. The enumerator asked the questions orally, clarified misunderstandings when necessary and recorded the respondent's answers in English by hand on the questionnaire sheet. The questionnaire contained a mix of open and closed questions. The questionnaire used in Paper I was not pre-tested prior to the study, but it was based on insights from semi-structured interviews and focus group discussions with farmers in the same area conducted earlier that year. The questionnaire used in Paper II, however, was pre-tested prior to the study. In Paper I, the questionnaire interviews were conducted by the same four people, and in Paper II, multiple people were involved in the interviews. They were all well-informed about the research purpose and proficient in both English and the local language of the district. Also, a member of the research team was present throughout the interviews and could therefore clarify any confusion.

3.5 Study area (Papers III and IV)

Paper III aimed to investigate the perceptions and practices of Zambian small ruminant traders on sheep and goat health and disease, and their potential impact on the risk for disease dissemination through trade. Paper IV aims to determine the seropositivity rates of selected zoonotic diseases in sheep and goats in trade, as well as risks for occupational exposure to these and other zoonotic diseases for slaughterhouse workers. Therefore, the two largest markets for small ruminants in Zambia were visited, namely the Lusaka and Kasumbalesa small livestock markets. The Lusaka market is situated in the Chibolya township in the capital, while the Kasumbalesa market is located close to a major border crossing-point to the DRC (Figure 5). The market activities are highly seasonal, with more trade taking place towards the end of the month, in connection with celebrations and holidays and prior to the due date of school fees. All market visits took place at times of relatively low market activity. In spite of this, the atmosphere at the market was often stressful, with pigs screaming and fighting, and loud negotiations between traders and potential buyers.

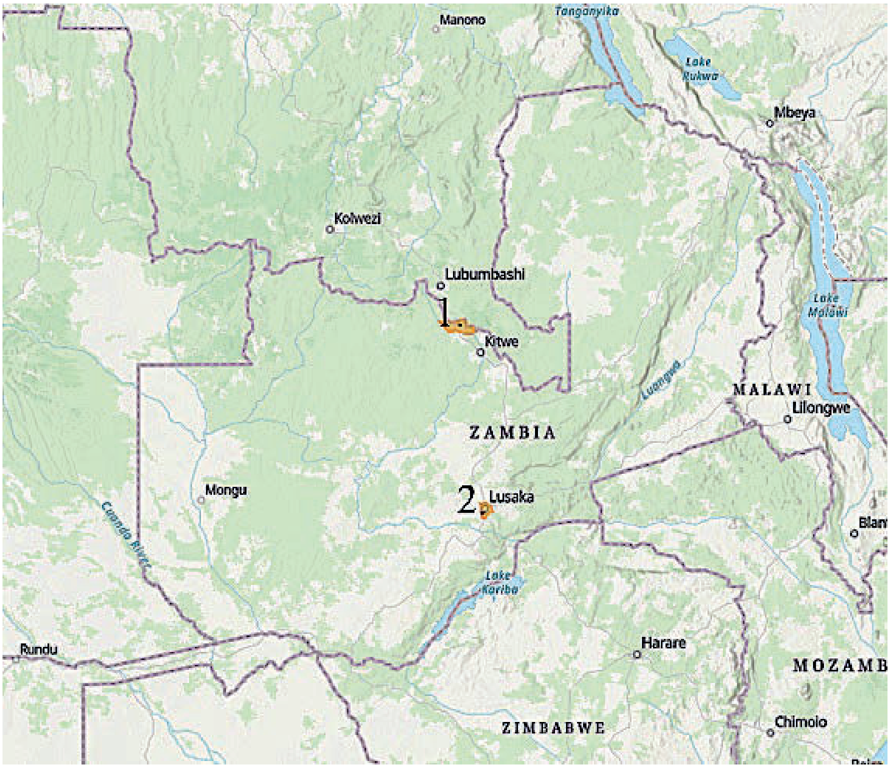

Figure 5: Map locations of the districts where the two surveyed markets are situated: 1=Chililalombwe district, containing the Kasumbalesa small livestock market, and 2=Lusaka district, containing the Lusaka small livestock market. Map kindly provided by Emma Lysholm. Source: Esri, USGS | Esri, © OpenStreetMap contributors, HERE, Garmin, FAO, NOAA, USGS. www.arcgis.com

The Lusaka and Kasumbalesa small livestock markets are informal and at the time of the study visits, they were run by the Small Livestock Association of Zambia (SLAZ). SLAZ is a non-governmental association whose primary purpose is to establish a more organised market system for small livestock in Zambia. At both markets, goats, pigs, sheep, chicken and other fowl were sold (Figure 6). Goats were considerably more common than sheep. In addition to animal pens, the Lusaka small livestock market also had a veterinary shop (i.e. a store where veterinary drugs can be purchased) and two slaughterhouses: one for small ruminants and one for pigs. At the Kasumbalesa market, there was no veterinary shop or designated slaughterhouse at the time of the study visits.

Figure 6a-c: a) Sheep in pens at the Lusaka market. b) Pigs and goats in pens at the Kasumbalesa market. c) Goats in a crowded pen at the Lusaka market. Photographs: Sara Lysholm

3.6 Study design (Papers III and IV)

Paper III was designed as a case study, which Gillham (2000) defines as a study of human activity embedded in the real world, which can only be understood if witnessed in its context. The case study of Paper III consisted of the Lusaka and Kasumbalesa small livestock markets. However, the fact that both markets were run by the same association, were structurally outlined in a similar manner, and many of the interviewed traders visited both markets, warrants the two markets being treated as one case. Paper IV employed a mixed-methods approach, combining quantitative sero-epidemiological data with qualitative data strategies (Conroy 2005).

In Paper III, the unit of analysis was small livestock traders and in Paper IV slaughterhouse workers. However, to enrich and triangulate the information that was offered by the traders and slaughterhouse workers, other value chain members present at the marketplace, including market customers, veterinarians, transporters etc., were also included (Yin 1994; Conroy 2005). The respondents were chosen using purposive sampling techniques (Conroy 2005), where informants who fulfil certain criteria, such as being a small ruminant trader, were targeted for inclusion in the research. The data collection for Paper III took place in April-May and September 2018. The visits to the Lusaka market were repeated until saturation was reached in the information from the traders, meaning that no new variations emerged in the collected data. At Kasumbalesa market, only four visits were made due to time constraints. By dividing the data collection over two field trips, a primary data analysis process could be conducted after field trip 1, where the generated data was critically evaluated. The insights were later used in preparation for the second field trip. The data collection for Paper IV was conducted simultaneously with that for Paper III. However, the field trip in September was dedicated to serological data collection and analysis, hence qualitative data was only obtained during the spring in 2018.

All data for Papers III and IV were collected with the same interpreter who had prior experience of working in research projects and was a skilled communicator. As the research findings were continuously being discussed, she provided important assistance in clarifying and contextualising the data. By working together for an extended period of time, she developed an understanding for what kind of information was of interest to the project, which enabled her to summarise the most important information from the respondents, which in turn ensured that the interviews ran smoothly.

3.6.1 Semi-structured interviews (Papers III and IV)

Semi-structured interviews (SSI) (Kruska *et al.* 2003) were the main mode of data collection for both Papers III and IV. While the SSI in Paper III were generally conducted individually, the SSI in Paper IV were typically held in groups. In both studies, a topic guide developed from the research questions was utilised to navigate the interviews, while at the same time remaining open to new and unexpected information supplied by the respondent (Gillham 2000). In Paper III, the topic guide mainly covered aspects such as the traders' definition of health and disease in sheep and goats, the actions they took if they discovered a sick small ruminant, and factors in the wider trading situation that are important for determining traders' actions in relation to animal disease. In Paper IV, the topic guide covered themes such as slaughter procedures, hygiene, and ante-mortem and post-mortem findings. Most interviews were conducted in the local languages of Nyanja or Bemba with an interpreter, and responses were noted down by hand in detailed field notes that were transcribed that evening (Southwold-Llewellyn 2002). In a small number of interviews, the interpreter and the respondent did not speak the same language, and on these occasions, other people present at the markets interpreted instead. These interviews were judged to be of lower quality because they were conducted with an untrained interpreter. This was taken into consideration later in the data analysis.

3.6.2 Focus group discussions and participatory ranking (Paper IV)

Two focus group discussions (Robson 2011) were performed with slaughterhouse employees in Lusaka. The purpose of these sessions was to complement and cross-check information from the SSI. One session was performed with supervisors and one with workers to avoid the potential power imbalance which can prevent free expressions of opinions out of fear of repercussions (Kapoor 2002; Fischer *et al.* 2020b). Both groups were convened by the slaughterhouse manager. The supervisor group consisted of five respondents throughout the session, whereas the worker group started with eleven participants, but they were then joined by several temporary participants for parts of the discussion, taking the number of participants up to a maximum of 20.

The focus group discussions were divided into two sections:

1) a group interview based on a topic guide that followed the same themes as the individual interviews, i.e. slaughter routines, procedures, hygiene, and ante-mortem and post-mortem findings
2) ranking exercises, where the participants were asked to list important aspects related to a number of themes, such as good slaughter hygiene, and subsequently rank the different listed aspects in order of importance.

The discussions were held in Nyanja, the local language, and were facilitated by a moderator. The moderator followed the topic guide while being open to following up any new information provided by the participants. I was present during both focus groups and took notes from the discussions, which were translated to me by the facilitator. Notes were also taken by an assistant note-taker who is fluent in Nyanja. These notes were transcribed later that day.

3.6.3 Observations (Papers III and IV)

One each visit to the Lusaka and Kasumbalesa markets, time between interviews was spent observing the ongoing market activities. For Paper IV, five sessions of observations were conducted at the slaughterhouse, generally lasting for one to two hours. In addition, several shorter observation sessions were conducted. The observations were used to complement the SSI and FGD data, as they revealed information that was not explicitly stated in the interviews or discussions, and aspects that were beyond verbal descriptions. The observations were noted down in field notes that were transcribed later that day. The observations focused on documenting slaughter procedures and identifying steps at which meat and organ hygiene was compromised or where the slaughterhouse workers were put at risk of occupational exposure to pathogens.

3.7 Qualitative data coding and analysis (Papers III and IV)

All field notes were later thematically coded (Miles & Huberman 1994) using the NVivo software (QSR International, Warrington, UK), a process that started towards the end of the first field trip. The coding process was inspired by a grounded theory approach, in which data collection and analysis occur concurrently and data concepts emerge from the data (Strauss & Corbin 1990). While the coding was guided by the research questions, it was open to new emerging themes. The process was iterative, and through repeated readings of the material (Miles & Huberman 1994; Bowen 2006) and by theoretically informing the data analysis (Bowen 2006), more detailed themes were found in the initial broad themes of the research questions.

3.8 Rates of seropositivity at Lusaka and Kasumbalesa small livestock markets (Paper IV)

The seroepidemiological part of Paper IV was designed to provide a cross-sectional view of the seropositivity rate of *Brucella* spp., *Coxiella burnetii* and RVFV in small ruminants present at Lusaka and Kasumbalesa markets. The study was based on a convenience sample scheme and the selection of animals to sample was non-random, as it was the trader who chose the sheep and goats from which samples could be taken. This was generally a prerequisite for the traders to grant their permission. A sample size was calculated prior to the study, but due to time constraints, the estimated number could not be met. Therefore, the aim shifted to collecting as many samples as the traders would allow us to, and to estimate seropositivity rate rather than seroprevalence. At the Lusaka market, serum samples were collected on three occasions over two weeks, while samples were collected only once at the Kasumbalesa market. After collection, the serum samples were placed in a vertical position in a cooler box to coagulate and allow serum to separate. Later that evening, the serum was transferred to cryotubes and stored at -20 °C until transportation to the laboratory, where samples were stored at -80 °C until analysis.

3.9 Serological analysis (Papers I, II and IV)

For the serological analysis, commercially available enzyme-linked immunosorbent assays (ELISA) were used. ELISA was chosen in preference to other serological methods as it is a relatively simple, quick, cost-efficient and robust procedure that is suitable for analysing large quantities of samples, and because of the high sensitivity and specificity of the utilised assays. The following kits were used: *ID Screen PPR competition ELISA* (ID-vet, France), *IDEXX CCPP Ab test* (IDEXX, The Netherlands), *ID Screen FMD NSP competition* (ID-vet, France), *ID Screen Capripox Double Antigen Multi-species* (ID-vet, France), *ID Screen Rift Valley Fever Competition Multi-species* (ID-vet, France), *ID Screen CCHF Double Antigen Multi-species* (ID-vet, France) and *ID Screen Q-Fever Indirect Multi-species* (ID-vet, France). All are competitive ELISAs, except for the Capripox and CCHF ELISAs which are double-antigen ELISAs, and the Q-fever ELISA which is an indirect ELISA. The Capripox ELISA detects antibodies for the Capripoxviruses SPPV, GTPV, and lumpy skin disease virus (LSDV). For brucellosis, two different kits were used as all samples in Zambia were analysed using *Svanovir Brucella-Ab C-ELISA* (Boehringer-Ingelheim Svanova diagnostics, Sweden), whereas in Tanzania, the LTELISA *Brucella* competitive ELISA kit, (LT Biotech, Lithuania) was utilized. Both kits detect antibodies for *B. melitensis*, *B. abortus* and *B. suis*. In Paper I, the *Brucella* spp., analysis in Tanzania was conducted more than one year after the others and at that time, a large number of samples could no longer be located. Hence, the number of analysed samples for *Brucella* spp., in Tanzania is lower compared to the other pathogens included in Paper I.

All kits were used and interpreted according to the manufacturers' instructions. The sensitivity and specificity values of the individual assays were obtained from the manufacturer. In addition, the literature was scanned for independent studies on the tests' analytical performance.

3.10 Statistical analysis (Paper I, II and IV)

In Papers I and II, true prevalence, and in Paper IV, the true seropositivity rate, were calculated using the apparent prevalence as well as the sensitivity and specificity of the respective ELISA, in accordance with Rogan and Gladen (1978). Univariable and multivariable statistical analyses were performed using Stata IC (StataCorp LLC, USA). In Paper IV, only univariable analysis was conducted as a maximum of one risk factor per pathogen was significant or close to significant ($p < 0.25$). As the traders only had limited information about each animal, the included risk factors were market, species, sex and provincial origin. The analyses were performed using Chi2 test or Fischer's exact test where applicable. Odds ratio (OR) was calculated for the significant predictor variables using logistic regression.

In Papers I and II, univariable and multivariable analyses were conducted on both animal-level and herd-level data. A herd was considered seropositive for a pathogen if one or more of the sampled animals in the herd tested positive. Predictor variables included in the animal-level analyses were for example age, sex, and species. In herd-level analyses, trade routines, contact frequency with sheep and goats from other herds, herd size, border proximity etc. were included. Univariable analysis was conducted using the Chi2 test or Fischer's exact test where applicable.

Logistic regression was performed to estimate odds ratios for variables that were not included in the multivariable analysis. For example, in Paper II, the seroprevalence in districts situated at an international border was compared with inland districts using logistic regression, while in the multivariable analysis, the individual districts were included instead. This was because the seroprevalence differed considerably between districts within the same category, indicating that the seroprevalence was more dependent on the district itself rather than on its proximity to an international border.

Multilevel mixed-effects logistic regression analysis was guided by directed acyclic graphs and performed using the meqrlogit command in Stata. Using a mixed-effects model allowed the inclusion of variables of primary interest as fixed (measurable), while random variables were included to account for their potential unmeasured clustering effect. In Paper I, included random variables were district, village, and herd in the animal-level analysis, and district and village in the herd-level analysis (in that hierarchical order, as village is nested within district, herd within village, and animal within herd). In Paper II, the same random variables were used except for district, which was included as a fixed variable.

All variables with a p-value of 0.25 or less in the univariable analysis were included in the multi-level mixed-effects logistic regression analysis, unless multicollinearity was detected, in which case the least relevant variable was excluded. Multicollinearity was tested for in all the models using variance inflation factor (VIF) and a cut-off value of ten was used. For example, in Paper I, strong collinearity was identified between country and grazing strategy and therefore, grazing strategy was removed from the models. In both Paper I and II, a select number of predictor variables were always included in the models as these were of special interest for the scope of the studies. In Paper I, the time of the latest introduction of a new sheep or goat, the last time a small ruminant was sold, and proximity to an international border, a town and the Tan-Zam highway were always included in the initial model. In Paper II, the frequency of purchasing and selling small ruminants was always included in the initial model. Initially, the full model was run, and the variable with the highest conjoined p-value using the Wald Test was removed in a stepwise backward elimination procedure. This continued until only one variable remained. Confounding was controlled for in each step, and a variable was judged to be a confounder when it affected the coefficient of other models with $\geq$ 20%. Selection of the best-fitting model was subsequently performed using the Akaike information criterion (AIC) and the likelihood ratio (LR) test. Also, residual plots were examined visually according to Dohoo *et al.* (2003). A p-value of <0.05 was considered statistically significant, but higher p-values were also presented in the results to avoid excluding potential associations between a variable and seropositivity or seronegativity.

3.11 Selected important ethical considerations (Papers I-IV)

In Papers I and II, the head of the district veterinary staff in each district was personally contacted and informed about the studies. In Papers III and IV, visits were made to representatives of the organisation in charge of the daily operations at the markets, including the head supervisor of the slaughterhouse. They were informed about the research aims and asked for consent for research activities to take place. Prior to data collection, each individual (human) participant was informed about the research purpose, and they were given the opportunity to ask any questions they might have. All participants were informed that participation was voluntary and could be interrupted at any time, without repercussions. In Papers I and II, written consent was obtained, whereas in Papers III and IV oral consent was used. Care was taken to ensure participant anonymity and confidentiality, e.g. by limiting the collection of personal details and restricting access to research data to members of the research team. The data included in Papers I and II were collected in collaboration with local veterinary personnel, and they were essential to gaining the farmers' trust and ensuring their cooperation. By spending extended periods of time at the markets and the slaughterhouse in Papers III and IV, trusting relationships could be established with the respondents. Carrying a notebook that was visible at all times, during SSI, FGD and observations, served to remind participants that they were being observed as part of a research project.

After completing the studies outlined in Papers I and II, the serological results were distributed to the individual farmers and to the local veterinary personnel. In the questionnaire interviews, several farmers expressed their frustration that when samples had been collected from their animals on previous occasions, they had never been informed of the results.

4. Results and discussion

A selection of the results is presented and discussed in this section. For a full report of the results, please refer to Papers I-IV.

4.1 Seroprevalence of selected transboundary diseases in Zambia and Tanzania (Papers I and II)

4.1.1 Descriptive statistics (Papers I and II)

For Paper I, 977 serum samples from sheep and goats from 324 herds were collected. Of these, 49.7% were obtained in Zambia and 50.3% in Tanzania. The samples were tested for the presence of antibodies to PPR, FMD, SGP, RVF and brucellosis. For Paper II, 962 goats from 280 different herds were sampled. Approximately 50.3% were sampled in a district with at least one international border, and the remaining 49.7% in an inland district. The samples were analysed for presence of antibodies to CCPP, FMD, RVF, CCHF and brucellosis. In Paper I, seven farmers in Tanzania had vaccinated their small ruminants for CCPP earlier in the same year. No vaccination was reported by the farmers in Paper II.

4.1.2 Apparent seroprevalence (Papers I and II)

The apparent animal-level seroprevalences are illustrated in Table 3, and herd-level seroprevalences in Table 4. Most of the sampled animals and herds were seronegative for all the included pathogens, both in Paper I (88.3% and 69.1% respectively) and Paper II (70.3% and 50.0% respectively). However, some animals had seroconverted for two or more pathogens (Paper I: 5.22% and Paper II: 3.53%).

Table 3: Apparent animal-level seroprevalence in percent, with confidence intervals at 95% presented in parentheses

	2018 (Zambia-Tanzania)			**2019 (Zambia)**		
	Total (%)	**Zambia (%)**	**Tanzania (%)**	**Total (%)**	**Border districts (%)**	**Non-border districts (%)**
PPR	1.54 (0.86-2.52)	0.21 (0.01-1.14)	2.85 (1.57-4.74)	*Confirmatory analyses ongoing*	*Confirmatory analyses ongoing*	*Confirmatory analyses ongoing*
CCPP	*not done*	*not done*	*not done*	10.1 (8.25-12.2)	3.10 (1.74-5.06)	17.2 (13.9-20.8)
FMD	9.02 (7.29-11.0)	1.03 (0.33-2.39)	16.9 (13.7-20.5)	10.9 (9.01-13.1)	4.13 (2.54-6.31)	17.8 (14.5-21.5)
SGP	0.10 (0.00-0.57)	0 (0-0.76)†	0.20 (0.01-1.13)	*not done*	*not done*	*not done*
RVF	2.76 (1.83-4.00)	2.26 (1.14-4.01)	3.26 (1.87-5.24)	0.21 (0.02-0.75)	0.42 (0.05-1.50)	0 (0-0.77)†
Brucellosis	6.71 (4.93-8.87)	1.65 (0.71-3.22)	20.0 (14.5-26.5)	8.97 (7.24-11.0)	12.2 (9.43-15.5)	5.67 (3.77-8.15)
CCHF	*not done*	*not done*	*not done*	3.43 (2.37-4.78)	1.65 (0.72-3.23)	5.23 (3.41-7.62)

†One-sided 97.5% confidence interval

Table 4: Apparent herd-level seroprevalence in percent, with confidence intervals at 95% presented in parentheses

	2018 (Zambia-Tanzania)			**2019 (Zambia)**		
	Total (%)	**Zambia (%)**	**Tanzania (%)**	**Total (%)**	**Border districts (%)**	**Non-border districts (%)**
PPR	4.01 (2.15-6.76)	0.63 (0.02-3.43)	7.32 (3.84-12.4)	*Confirmatory analyses ongoing*	*Confirmatory analyses ongoing*	*Confirmatory analyses ongoing*
CCPP	*not done*	*not done*	*not done*	17. 1 (12.9-22.1)	8.12 (4.40-13.5)	29.2 (21.2-38.2)
FMD	18.6 (14.5-23.3)	3.14 (1.03-7.19)	33.5 (26.4-41.3)	22.1 (17.4-27.5)	11.9 (7.30-17.9)	35.8 (27.3-45.1)
SGP	0.31 (0.01-1.71)	0 (0-2.28)†	0.61 (0.02-3.35)	*not done*	*not done*	*not done*
RVF	7.41 (4.80-10.8)	5.62 (2.60-10.4)	9.15 (5.21-14.6)	0.71 (0.09-2.56)	1.25 (0.15-4.44)	0 (0-3.03)†
Brucellosis	14.4 (10.0-19.6)	5.00 (2.18-9.61)	38.1 (26.1-51.2)	18.2 (13.9 – 23.2)	22.5 (16.3-29.8)	12.5 (7.17-19.8)
CCHF	*not done*	*not done*	*not done*	8.93 (5.86-12.9)	3.75 (1.39-7.98)	15.8 (9.81-23.6)

†One-sided 97.5% confidence interval

4.1.3 Seroprevalence of PPRV (Paper I)

In Paper I, the animal-level and herd-level seroprevalence in Tanzania was 2.85% (95% CI 1.57-4.74) and 7.32% (95% CI 3.84-12.4). The seroprevalence in goats was 2.85% (95 % CI 1.57-4.74) while none of the 27 sampled sheep tested positive. These results were low compared with previous studies, where animal-level seroprevalence has ranged from 8.20-45.5% in sheep and 10.8-49.5% in goats (Swai *et al.* 2009; Karimuribo 2011; Muse *et al.* 2012; Kivaria *et al.* 2013; Mbyuzi *et al.* 2014; Kgotlele *et al.* 2016; Torsson *et al.* 2017; Herzog *et al.* 2019). Most of these studies were conducted in the northern parts of Tanzania, which together with differences in study design, the laboratory test used and surveyed years, at least in part can explain the differences in seroprevalence compared with Paper I. However, in a study by Chota *et al.* (2019) Mbozi district was included, of which both Tunduma and Momba districts were part when the study was conducted in 2016-2017. In this study, 17% of the 100 goats and 52.2% of the 23 sheep tested positive for presence of PPR-specific antibodies (Chota *et al.* 2019). The study was conducted following a vaccination campaign in the area that unfortunately had to be terminated in 2013 due to a lack of funding (E. S. Swai, personal communication). As there is no DIVA vaccine that enables differentiation between seroconversion following natural infection and vaccination, and since the vaccine generally gives rise to life-long immunity (Mariner *et al.* 2017), the vaccination campaign can have contributed to the higher seroprevalence observed by Chota *et al.* (2019). In Paper I, none of the participating farmers reported their animals having been vaccinated for PPRV. As a result of the previous vaccination campaign, the infectious pressure in the area may have been reduced. This, together with the short population turnover rate in small ruminants (Otte & Chilonda 2002), may explain the low seroprevalence detected. Furthermore, the fact that none of the seropositive animals were less than a year old, and only three seropositive animals were three years old or less, can indicate limited viral activity in the area in recent years.

While PPRV is endemic in Tanzania, the virus has yet to be detected in Zambia. So far, only antibodies without connection to clinical disease have been found in the country (OIE 2016). In Paper I, only one goat of approximately two years of age tested positive for antibodies to PPRV.

This corresponds to an animal-level seroprevalence of 0.21% (95% CI 0.01-1.14). The seropositive animal had not been vaccinated and was reported to have been born into the herd. While the seropositive goat in this study tested positive using two separate ELISA kit batches, this result needs to be verified with another methodology, preferably the virus neutralisation test (VNT) or similar. Furthermore, to confirm presence of PPRV in Zambia, the detection of the virus or viral genome would be required.

4.1.4 Seroprevalence of Mccp (Paper I)

Mccp was included in Paper II, which is the first report of the presence of antibodies to CCPP in goats in Zambia. The animal-level and herd-level seroprevalence detected were 10.1% (95% CI 8.25-12.2) and 17.1% (95 % CI 12.9-22.1) respectively. Seropositive goats were detected in all surveyed districts and age groups, which indicates that Mccp is circulating in the country. None of the farmers reported that their goats had been vaccinated to CCPP, and according to veterinary personnel in the area, no vaccination campaign had been conducted.

4.1.5 Seroprevalence of FMDV (Papers I and II)

FMDV is endemic in both Zambia and Tanzania (Picado *et al.* 2011; Hamoonga *et al.* 2014; Sinkala *et al.* 2014a; Allepuz *et al.* 2015), and the virus was included in both Papers I and II. These studies are the first that investigates the seroprevalence of FMDV in small ruminants in Zambia. Unfortunately, no attempt was made to type the serotype of FMDV present in either Paper I or II, but this would be interesting for future studies.

In Paper I, the seroprevalence was significantly higher in Tanzania, with animal-level and herd-level seroprevalence estimated to be 16.9% (95% CI 13.7-20.5) and 33.5% (95% CI 26.4-41.3) in Tanzania, and 1.03% (95% CI 0.33-2.39) and 3.14% (95% CI 1.03-7.19) in Zambia. The animal-level seroprevalence in goats was 8.85% (95% CI 7.12-10.8) and in sheep 14.8% (95% CI 4.19-33.7), however, as only 27 sheep were sampled, this result should be interpreted with caution. The detected seroprevalence was surprisingly low in both Zambia and Tanzania, as the border region has repeatedly been identified as a hotspot area for FMD outbreaks in cattle (Picado *et al.* 2011; Hamoonga *et al.* 2014; Sinkala *et al.* 2014a; Allepuz *et al.* 2015). The fact that the aerosol production of FMDV in small ruminants is comparatively small (Kitching 2005) and their population turnover rate is high compared with cattle (Otte & Chilonda 2002) are potential explanations for the low seroprevalence detected. Therefore, small ruminants could indeed play a role in the epidemiology of FMDV in other species, including cattle, in the region.

In Paper II, the detected animal-level and herd-level seroprevalence in goats in Zambia was 10.9% (95% CI 9.01-13.1) and 22.1% (95% CI 17.4-27.5) respectively. Seropositive goats were found across the country and in all age groups, indicating widespread occurrence and active circulation of the virus. However, the fact that the seroprevalence varied in different areas indicated geographical clustering. The result from Paper II was considerably higher than in Paper I, however as the two studies were conducted in different years and areas of the country, comparisons should be made with caution. Furthermore, there was an ongoing outbreak of FMD in two of the surveyed districts included in Paper II (Mazabuka and Monze) at the time of sample procurement. Excluding these two districts from the analysis led to detected animal-level and herd-level seroprevalence of 3.88% (95% CI 2.53-5.68) and 12.0% (95% CI 7.84-17.3) respectively. While this seroprevalence is lower, it is still higher than that found in Paper I. Also, in Paper I, no seropositive animals under one year of age was found, indicating limited viral activity in the surveyed region during the year preceding the study. This is surprising given that the surveyed region in Paper I is considered a hotspot area.

4.1.6 Seroprevalence of SGPV (Paper I)

While SGPV genetic material has been found in Tanzania (Kgotlele *et al.* 2019) and the DRC (Birindwa *et al.* 2017), it has yet to be detected in Zambia. No seroprevalence studies in Zambia, Tanzania or any of the neighbouring countries have previously been conducted. In Paper I, only one two-year old goat in Tanzania tested positive for the presence of antibodies to SGPV, corresponding to an animal-level seroprevalence of 0.20% (95% CI 0.01-1.13). None of the sampled animals in Zambia tested positive. The ELISA that was used in Paper I also detects antibodies to lumpy skin disease virus (LSDV), which has been detected in the region previously (Mweene *et al.* 1996). While experimental infection of LSDV in sheep and goats has been reported (Namazi & Khodakaram Tafti 2021) there is no evidence of this occurring naturally, and the risk is therefore low that the seropositive goat in this study had seroconverted following exposure to LSDV. Due to the potential of a false positive result, more research is needed to elucidate whether SGPV is present and circulating in small ruminants in the Tanzania-Zambia border region.

4.1.7 Seroprevalence of RVFV (Papers I and II)

RVFV is endemic in both Zambia and Tanzania, but no outbreak has been reported in Zambia since 1989 (Dautu *et al.* 2012) and in Tanzania since 2007 (Sindato *et al.* 2014; Ahmed *et al.* 2018). In Paper I, the animal-level and herd-level seroprevalence 3.26% (95% CI 1.87-5.24) and 9.15% (95% CI 5.21-14.6) in Tanzania and 2.26% (95% CI 1.14-4.01) and 5.62% (95% CI 2.60-10.4) in Zambia. The seroprevalence in goats was 2.74% (95% CI 1.80-3.98), while one of the 27 sheep tested positive, corresponding to a seroprevalence of 3.70% (95% CI 0.09-19.0). Seropositive animals were detected in all age groups and therefore these results indicated inter-epidemic circulation of RVFV in the area. Indications of this has previously been found in the SSA region, for example in Kenya (Mbotha *et al.* 2018), Zambia (Davies *et al.* 1992; Saasa *et al.* 2018) and Tanzania (Sumaye *et al.* 2013; Wensman *et al.* 2015).

The results from Tanzania in Paper I were slightly lower compared with previous studies conducted during inter-epizootic periods, where animal-level seroprevalence has ranged from 7.70-12.5% in sheep and 4.70-12.5% in goats (Sumaye *et al.* 2013; Kifaro *et al.* 2014; Wensman *et al.* 2015). These studies were conducted four (Sumaye *et al.* 2013; Kifaro *et al.* 2014) and seven years (Wensman *et al.* 2015) after the most recent reported epizootic outbreak. Particularly in the studies of Sumaye *et al.* (2013) and Kifaro *et al.* (2014), but also in the study of Wensman *et al.* (2015), it is possible that some of the sampled animals were alive and seroconverted during the outbreak. In Zambia, the RVF seroprevalence in livestock has in previous studies generally been investigated following an epizootic outbreak (Hussein *et al.* 1985; Morita 1988; Ghirotti *et al.* 1991; Davies *et al.* 1992; Samui *et al.* 1997). In a more recent study conducted by Saasa *et al.* (2018), the seroprevalence in cattle in 2014, during an inter-epidemic period, was estimated to 5.4%, which is similar to the result in Paper I.

In Paper II, the seroprevalence was considerably lower than in Paper I, as only two animals tested positive, which equals an animal-level and herd-level seroprevalence of 0.21% (95% CI 0.02-0.75) and 0.71% (95% CI

0.09-2.56) respectively. Although also these seroconversions could have been due to inter-epidemic circulation of RVFV, the possibility of false positive laboratory results must not be excluded. One potential explanation for the difference in seroprevalence in Zambia between Papers I and II is the difference in sample regions. As the northern parts of the country receive more rainfall (Makondo & Thomas 2020), it is possible that the activity of the mosquito vector is higher in this region.

4.1.8 Seroprevalence of CCHFV (Paper II)

The presence of antibodies to CCHFV was included in Paper II. The detected animal-level and herd-level seroprevalence was 3.43% (95% CI 2.37-4.78) and 8.93% (95% CI 5.86-12.9) respectively. Seropositive animals were found in all age groups and in all districts except one, indicating viral circulation in large areas of the country. The result can be compared to a recent study on cattle in Zambia, where an 8.4% animal-level seroprevalence was found (Kajihara *et al.* 2021). In addition to differences in surveyed areas and years as well as in study design and methodology, the higher seroprevalence in cattle can be caused by an in general larger *Hyalomma* spp. tick burden in this species (Spengler *et al.* 2016).

4.1.9 Seroprevalence of *Brucella* spp. (Papers I and II)

Brucella spp. is endemic in both Zambia and Tanzania. In Paper I, the seroprevalence in goats was 6.89% (95% CI 5.07-9.11), while none of the 18 analysed sheep samples tested positive. The detected animal-level and herd-level seroprevalence was 20.0% (95% CI 14.5-26.5) and 38.1% (95% CI 26.1-51.2) in Tanzania, and 1.65% (95% CI 0.71-3.22) and 5.00% (95% CI 2.18-9.61) in Zambia. The seroprevalence was significantly higher in Tanzania than Zambia, however as the ELISA kits used differed between the two countries, comparisons should be made with caution. While the low seroprevalence in Zambia is in line with findings in a previous study Muma *et al.* (2006), the detected seroprevalence in Tanzania is considerably higher than in previous reports, where results have ranged from 0-1.6% (Assenga *et al.* 2015; Shirima & Kunda 2016).

In Paper II, the detected seroprevalence in Zambia was considerably higher than that found in Paper I, with an animal-level and herd-level seroprevalence of 8.97% (95% CI 7.24-11.0) and 18.2% (95% CI 13.9-

23.2) respectively. As the surveyed year and regions differed between Papers I and II, comparisons should be made with caution. Seropositive animals were found in all surveyed districts and in all age groups, which is line with the bacteria's endemic status. The seroprevalence in Siavonga district was significantly higher than in the other visited districts, with an animal-level and herd-level seroprevalence of 41.7% (95% CI 32.7-51.0) and 72.5% (95% CI 56.1-85.4) respectively. If the results from Siavonga are excluded from the seroprevalence calculation, animal-level and herd-level results were 4.29% (95% CI 3.02-5.89) and 9.17% (95% CI 5.83-13.5) respectively, i.e. more in line with the detected results in Paper I.

4.2 The impact of selected predictor variables on seroprevalence (Papers I and II)

The association between predictor variables (or risk factors and protective factors) and seroprevalence was calculated on both animal-level and herd-level data. In general, the analyses identified the same variables as risk factors or protective factors in both animal-level and herd-level data. A selected few of these predictor variables are presented here. For a full list, please see Papers I and II and the supplementary materials of the respective papers. Unless otherwise specified, the results presented here are herd-level predictor variables.

4.2.1 The impact of proximity to an international border on seroprevalence (Papers I and II)

In previous studies performed in Zambia and Tanzania, outbreaks of FMD in cattle have been found to be clustered along several international borders, including the Tanzania-Zambia border (Perry & Hedger 1984; Picado *et al.* 2011; Hamoonga *et al.* 2014; Sinkala *et al.* 2014a; Allepuz *et al.* 2015). One probable explanation for this is that animal trade and movement tend to increase close to some international borders (Di Nardo *et al.* 2011). In this thesis, the aim was to investigate if border proximity had an impact on the seroprevalence of selected transboundary diseases in small ruminants.

Paper I investigated whether the seroprevalence differed between herds situated 10 km or less (≤10 km), more than 10 km to 30 km (>10-30 km) or more than 30 km (>30 km) from an international border. The results were surprising, as a positive association between seroprevalence and border proximity was only observed for brucellosis. For brucellosis, herds situated ≤10 km (OR 4.05, 95% CI 0.94-17.4, p=0.060), or >10-30 km (OR 4.43, 95% CI 1.22-16.1, p=0.024), from an international border were more likely to be seropositive, although the difference was only statistically significant for the >10-30 km group. For PPR (OR 6.83, 95% CI 1.37-34.0, p=0.019) and FMD (OR 5.68, 95% CI 1.58-20.3, p=0.008), however, the seroprevalence was significantly higher in households situated 30 km or more from a border. The same tendency was seen for RVF, although the association was not statistically significant (OR 2.54, 95% CI 0.86-7.53, p=0.093).

In Paper II, the seroprevalence in districts situated at one or more international borders was compared with inland districts in univariable analysis, while the individual districts were included in the multivariable analysis. In the univariable analysis, the rate of seropositivity in herd-level data was significantly higher in inland districts for CCPP (OR 4.66, 95% CI 2.33-9.29, p<0.001), FMD (OR 4.14, 95% CI 2.26-7.61, p<0.001) and CCHF (OR 4.83, 95% CI 1.86-12.5, p=0.001). For RVF, both seropositive animals originated from districts with international borders, but the difference was not statistically significant (p=0.508), while for brucellosis, the seroprevalence was significantly higher in districts that have an international border (OR 2.03, 95% CI 1.05-3.92, p=0.034). In the multivariable analysis, the seroprevalence in all the surveyed inland districts except Chibombo was significantly higher for at least one pathogen, namely FMD (OR 22.7, 95% CI 3.62-143, p=0.001) in Monze district, and CCPP (OR 987, 95% CI 7.45-130794, p=0.006), brucellosis (OR 9.55, 95% CI 1.39-65.4, p=0.022) and CCHF (OR 46.1, 95% CI 2.58-823, p=0.009) in Mazabuka district. In the districts situated at an international border, however, only the seroprevalence of brucellosis (OR 110, 95% CI 13.9-865, p<0.001) was significantly higher in Siavonga district.

Hence, the findings in Papers I and II differed considerably from the results of previous studies. The earlier studies analysed the spatio-temporal nationwide distribution of reported FMD outbreaks in cattle. In Paper I, all the herds that participated were situated within a 90 km radius of an international border. Therefore, an outbreak of a disease in any of these herds would probably have been identified as having occurred in proximity to a border in a spatial analysis. In Paper II, the surveyed inland districts were all situated close to the capital Lusaka and in areas of the country with the largest small ruminant populations (Ministry of Fisheries and Livestock 2019). Furthermore, long distance trade and movement are more common in these areas and farmers are generally considered to be business oriented and likely to engage in trade (Lubungu *et al.* 2012; Namonje-Kapembwa *et al.* 2016; Chapoto & Subakanya 2019). This can contribute to the higher seroprevalence observed in these areas compared to the border districts, which were more remote and less densely populated by both humans and small ruminants.

It is noteworthy that in both studies, brucellosis is the only disease where the ratio of seropositivity was higher close to international borders. In Paper I, the vast majority of the seropositive animals were found in Tanzania. Unfortunately, less than 40% of the Tanzanian samples could be analysed for presence of antibodies to *Brucella* spp. Among these samples, disproportionately few were situated more than thirty kilometres from the border, which may have affected the result. In Paper II, the higher seroprevalence in border districts was likely affected by the very high seroprevalence in Siavonga district, where animal and herd-level seroprevalence was 41.7% and 72.5% respectively. Excluding Siavonga district from the analysis, the animal and herd-level seroprevalence in districts with an international border was significantly lower than that of the inland districts, indicating a negligible impact of border proximity on the seroprevalence of brucellosis also in Paper II.

4.2.2 The impact of trade on seroprevalence (Papers I and II)

Trade is a well-known risk factor that has been associated with the dissemination of multiple pathogens, including FMD (Mansley *et al.* 2003; Ortiz-Pelaez *et al.* 2006; Robinson & Christley 2007), PPR (Kivaria *et al.* 2013) and RVF (Sherman 2011). In Paper I, a non-significant association between herd seropositivity for FMDV and buying sheep and goats at markets or from traders was identified (OR 2.47, 95% CI 0.95-6.43, p=0.065). At livestock markets, animals are often kept under stressful conditions in close proximity, facilitating pathogen exposure and spread (Naguib *et al.* 2021). Traders often move between villages in search of animals to buy and later resell, thereby mixing animals from different herds and villages (as was found in Paper III), thereby potentially contributing to the dissemination of animal diseases.

Frequent introduction of new animals into a herd has been associated with increased seroprevalence of, for example, brucellosis (Asmare *et al.* 2013; Nthiwa *et al.* 2019) and FMD (Osmani *et al.* 2021) in previous studies. However, in Papers I and II, no associations between seroprevalence and the time of the latest introduction of an animal into the herd (Paper I) or frequency of purchasing new animals (Paper II) was identified for most of the diseases. In general, for both Papers I and II, it was rare for the small ruminant-keeping households visited to acquire new animals for the herd. The ones that did mostly bought them from other farmers in the village or from nearby villages. Most small ruminants were mainly grazed on communal grazing grounds and came into frequent contact with sheep and goats from other herds in the area. Therefore, they are likely to regularly come into contact with the potential pool of source animals from which the farmer would be buying, which could explain why acquiring new animals was not generally identified as a risk factor. The only exception was brucellosis. In Paper I, the seroprevalence of brucellosis was higher in herds that had **not** introduced new animals into the herd in the last year, although this association was non-significant (OR 3.72, 95% CI 0.96-14.4, p=0.057). In Paper II, the animal-level seroprevalence of brucellosis was higher in herds that bought new animals at least once every two years, compared to herds that either bought at least on a yearly basis or more rarely, although this association was not statistically significant (OR 4.26, 95% CI 0.72-25.1, p=0.109). While not having introduced new animals to the herd in the last year (Paper I) is not necessarily the same as not buying new animals frequently or at all (Paper II), one potential interpretation of the combined results is that the seroprevalence of brucellosis in herds where new animals are introduced more frequently is lower than in herds where this occurs more rarely. This could be the result of differences in biosecurity measures that aim to reduce the risk of introducing disease with new animals, such as quarantining new purchased animals for certain time periods. Unfortunately, the usage of biosecurity measures was not investigated in this thesis.

The impact of selling small ruminants also varied between the pathogens. In Paper II, selling animals twice a year or more was associated with increased seroprevalence of *Brucella* spp. (OR 4.13, 95% CI 1.07-

16.0, p=0.040). One possible explanation for this is that small ruminant farmers often sell to traders who move between herds and villages taking already purchased animals with them, allowing them to intermingle with local goats before the traders move on with what they have bought (Namonje-Kapembwa *et al.* 2016). Conversely, in Paper II, non-significant associations between selling more rarely than once every two years, or never, and increased seroprevalence for FMDV (2.88 0.99-8.43, p=0.053) in herd-level data, and Mccp (OR 3.90, 95% CI 0.85-17.9, p=0.080) in animal-level data, were found. It is possible that the farmers who had observed signs of these diseases in their animals were withholding them from sale for this reason, thus reducing the potential outflow of seropositive animals from the herd through trade.

4.2.3 Other selected risk factors and their impact on seroprevalence (Paper I)

In addition to the impact of border proximity and trade, the analyses identified a few other variables associated with seroprevalence that are worth highlighting. In Paper I, associations between proximity to a town and seropositivity for FMDV on herd-level data (OR 79.2, 95% CI 4.52-1389, p=0.003), and *Brucella* spp., on animal-level data (OR 9.68, 95% CI 2.17-43.2, p=0.003), were identified. In the Tanzania-Zambia border region, 'goat soup' (or 'supu ya mbuzi') is considered a delicacy and is often sold at local restaurants. Therefore, many farmers trek or transport their goats to a nearby town to sell them to a restaurant owner. As many restaurants have limited access to cold storage space, the goats are generally kept alive until the meat is needed. During this time, it is possible that the goats come into contact with local animals at e.g. communal pastures, enabling them to exchange infectious pathogens.

In an earlier study, clustering of FMD outbreaks in cattle along the Tan-Zam highway (Allepuz *et al.* 2015) was identified. Cattle are often moved along this road to markets or slaughterhouses in other parts of the country. Surprisingly, in Paper I, the detected seroprevalence of FMDV was higher in herds situated 30 km or more from this major infrastructural route, although this association was not statistically significant (OR 11.8, 95% CI 0.93-150, p=0.057). Compared with cattle, small ruminants are often sold locally (Namonje-Kapembwa *et al.* 2016), which possibly reduces the significance of a large trade route such as the Tan-Zam highway. However, this should be interpreted with caution, as the vast majority of the participating herds were situated more than 30 km from the highway, which may have biased the results.

4.3 The informal small ruminant market and trade system in Zambia, and potential implications for disease spread (Papers III and IV)

4.3.1 Small ruminant trade in Zambia and the potential for dissemination of disease (Papers III and IV)

At the two largest small ruminant markets in Zambia, namely the Lusaka and Kasumbalesa small livestock markets, small ruminants from different parts of the country were mixed and kept in close proximity. Most of the animals came from districts in Southern Province, followed by Eastern and Central Provinces (Figure 7). Most of the small ruminants sold were slaughtered for consumption, however, a small fraction was kept alive and transported to farms in different parts of the country. The markets were often visited by traders from other countries, mainly the DRC but also Angola. These traders would purchase animals and transport them back to their home countries, where they generally were slaughtered for consumption upon arrival, but some were kept alive and used for e.g. dowry payments. At the time of visits, there was no animal health surveillance system in place and hygiene conditions were poor, with limited access to water for maintaining personal hygiene and for cleaning.

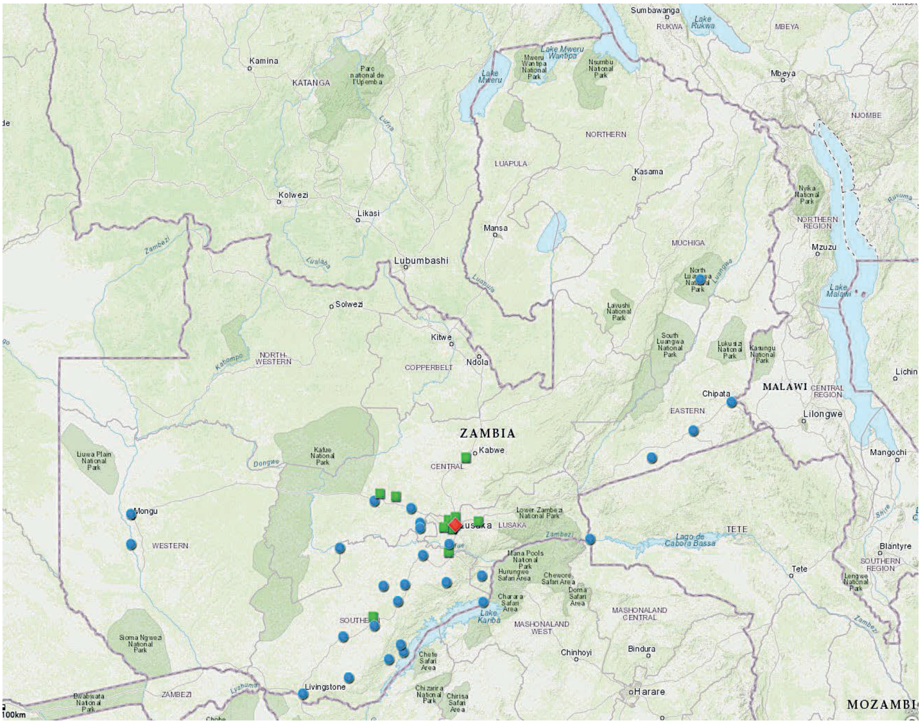

Figure 7: Schematic map indicating the origin (blue dots) of some of the sheep and goats going to the Lusaka market (red diamond), and where the ones that are kept alive end up after being sold (green squares). Source: Esri, USGS | Esri, © OpenStreetMap contributors, HERE, Garmin, FAO, NOAA, USGS. www.arcgis.com

4.3.2 Perceptions and practices of small ruminant traders related to the trade of sheep and goats with signs of disease (Paper III)

Most of the sheep and goat traders who participated in the study presented in Paper III either traded with small ruminants with clinical signs of disease or believed it to be more or less acceptable to do so, at least under certain conditions. Overall, the respondents' motivation for trading sick sheep and goats can be grouped into three different categories:

In the first category, the traders appeared unaware of the risks associated with selling sick small ruminants or unwilling to accept them as such, e.g. by avoiding to classify certain clinical signs as potential indications of disease. For example, many respondents stated that selling coughing sheep and goats was acceptable, as they viewed coughing as a result of different weather conditions rather than as a potential indication of infection by a pathogen. Furthermore, several traders were comfortable with selling sick sheep and goats for consumption, as they did not believe that consumption of small ruminants with clinical signs could result in human disease.

In the second category, the traders assigned responsibility of preventing disease transmission in trade elsewhere, most commonly to the veterinary authorities. In order to transport animals in Zambia, a veterinary certificate must be obtained prior to transport where the health of the animals is certified (Parliament of Zambia 1994; Parliament of Zambia 2010). This certificate should subsequently be checked regularly at veterinary checkpoints en route. Many traders therefore believed that the veterinary authorities were responsible for preventing the presence of sick animals in trade and at the markets. However, in Paper III, several shortcomings of the system were identified that reduces its ability to prevent transportation of sick animals. For example, the process for issuing the veterinary certificate varied considerably and, in some districts, it was reported that veterinarians would sign the papers without ever seeing the animals.

In the third category, the respondents were aware of the risks associated with selling sick small ruminants but did not see any other feasible alternatives than selling the animal. Some would attempt to sell the sick small ruminant quickly to avoid it succumbing to the illness before being sold, while others would slaughter it and sell the meat instead. These traders often expressed feelings of moral conflict, but were unable to withhold the animals from sale or transport them away from the market, most commonly due to financial limitations.

"*I know selling sick animals is wrong, but what can I do? I don't have the ability to treat them like I would if my goats at home got sick. I can't transport them back home because it's too expensive. My only option is to sell them to avoid losing money.*"

Trader at the Kasumbalesa small livestock market

4.3.3 Knowledge levels and risky behaviours (Paper III)

In previous studies, lack of knowledge is a commonly stated reason as to why traders engage in risky behaviours, such as trading sick animals (Neupane *et al.* 2012; Yu *et al.* 2013; Leslie *et al.* 2016; Elelu 2017). These studies often conclude that the traders should be educated about the associated risks as this would reduce their tendency to buy and sell sick animals. However, previous studies on farmers indicate that individual farmers' normative frames of reference (Jansen *et al.* 2009), attitudes on disease risks (Garforth *et al.* 2013), perceived effectiveness of control measures (Jansen *et al.* 2009; Garforth *et al.* 2013), sense of responsibility for preventing disease spread (Garforth *et al.* 2013) and financial and other structural constraints (Fischer *et al.* 2019) have a greater influence on how farmers act on animal disease than their factual knowledge. In Paper III, the respondent traders traded sick small ruminants for a wide variety of reasons, and the results indicate that only some traders would be significantly affected by a knowledge improvement campaign. Consequently, there is a clear need for researchers to move away from focusing on identifying and curing knowledge gaps. Other structural or procedural aspects that can contribute to the dissemination of disease in trade, as well as different limitations that prevent value chain actors to act in less risky ways, must also be acknowledged and included in future intervention strategies.

4.3.4 The 'good trader' (Paper III)

As people gain respect and social standing by abiding by norms embedded in their community culture (Burton 2004; Sutherland & Darnhofer 2012; Saunders 2016), it is relevant to study this aspect to understand why they reason and act the way that they do. The importance of understanding the concept of the model peer has been recognised and for example, it has been shown that 'good farmer' ideals have an impact on how farmers reason and behave (Burton 2004; Sutherland & Darnhofer 2012; Saunders 2016). Similarly, the findings in Paper III show that adhering to 'good trader' ideals was an important driver also for how the traders reasoned and behaved. However, the results indicate that the traders relate to a different culture and context than the farmers in studies on the cultural shaping of farmer behaviour. For example, while farmers often highlight the importance of establishing a relationship with their animals (Naylor *et al.* 2016; Shortall *et al.* 2017; Fischer *et al.* 2019), the traders in the Paper III emphasised the ability to build relationships with people, i.e. being good at dealing with clients. Hence, being a good trader has less to do with how animals are cared for and more to do with maintaining good customer relations. Therefore, influencing customer demands is likely to be an efficient way to adjust traders' perceptions and practices.

"A good trader is someone who can relate to customers and be good to them, even if they are rude back, and someone who keeps the environment clean to attract customers"

Trader at the Lusaka small livestock market

Some respondents also described a "good trader" as someone who keeps animals that look healthy. However, this was emphasised because it attracts clients and not because it is beneficial from an animal health perspective.

"*A good trader is someone who trades with animals that are clean and look fat and healthy, since this is what attracts customers*"

Trader at the Lusaka small livestock market

4.3.5 Seropositivity rates of selected zoonotic diseases in sheep and goats at the Lusaka and Kasumbalesa small livestock markets (Paper IV)

The seropositivity rates of the selected zoonotic pathogens were 10.1% (*Brucella* spp.), 5.91% (*Coxiella burnetii*) and 0.84% (RVFV) in sheep and goats at the livestock markets (Table 5). Only two animals tested positive for presence of antibodies to RVFV, and it cannot be ruled out that these animals are false positives.

Table 5: Seropositivity rates in percent, with 95% confidence interval in parentheses

	Total (%)	**Lusaka (%)**	**Kasumbalesa (%)**
***Brucella* spp.**	10.1 (6.60-14.7)	4.26 (1.17-10.5)	14.0 (8.76-20.8)
Coxiella burnetii	5.91 (3.27-9.71)	7.45 (3.05-14.7)	4.90 (1.99-9.83)
RVFV	0.84 (0.10-3.01)	0 (0-3.85)†	1.40 (0.17-4.96)

† One-sided confidence interval (97.5%)

These results were not surprising as all three pathogens have previously been detected in Zambia (Bell *et al.* 1977; Qiu *et al.* 2013) and no RVF outbreak has been reported in the last three decades (Dautu *et al.* 2012). The presence of *Brucella* spp. and *Coxiella burnetii* in the markets' source populations constitute occupational hazards for the workers at the Lusaka market slaughterhouse (FAO *et al.* 2006; Angelakis & Raoult 2010; Ikegami & Makino 2011). Furthermore, as *Brucella* spp. and RVFV can infect people through the consumption of undercooked meat (FAO *et al.* 2006; OIE 2019), it also constitutes a risk for the thousands of people who consume the meat and offal that leaves the studied markets.

4.3.6 Zoonotic disease risks at the Lusaka market slaughterhouse (Paper IV)

Considerable research has been conducted to investigate the working and hygiene conditions at slaughterhouses in low- and lower-middle-income countries around the world, including Sub-Saharan Africa (Nonga *et al.* 2010; Bello *et al.* 2011; Komba *et al.* 2012; Wamalwa *et al.* 2012; Ndalama *et al.* 2013; Cook *et al.* 2017a; Mpundu *et al.* 2019; Phiri *et al.* 2020). However, these studies have generally been conducted in the formal rather than the informal sector, and tend to focus on the slaughter of cattle, poultry and pigs, not small ruminants.

At the informal small ruminant slaughterhouse at the Lusaka small livestock market, the everyday operations at the slaughterhouse were performed by workers who worked under supervisors. The workers lacked formal education, which has been associated with increased bacterial contamination of carcasses in a previous study (Wamalwa *et al.* 2012). The workers generally commenced the slaughter procedure by hanging the animal upside down and slitting the throat to bleed it, without prior stunning. Following the bleeding, the slaughterhouse worker skinned the animal and opened up the abdomen and thorax to remove internal organs, without tying the oesophagus or colon prior to removal. To keep track of which body parts and organs came from which animal, the workers would generally place them on the inside of the pelt on the floor.

Multiple hygiene pitfalls and risks of occupational exposure and for contamination of meat and offal were observed in the slaughterhouse (Table 6 and Figure 8). For example, the workers did not have access to work-specific clothing or footwear, or to any personal protective equipment such as plastic gloves or aprons, which in previous studies has been associated with an increased risk of exposure to *Brucella* spp. (Nabukenya *et al.* 2013; Esmaeili *et al.* 2016; Awah-Ndukum *et al.* 2018) and *Leptospira* spp. (Cook *et al.* 2017b). The workers mainly used two different tools when conducting their work, i.e. a knife and a bucket containing water. This water was used to clean intestines of faecal material by dipping them in the water and letting it rinse through, and as a result, the water was often contaminated by faecal matter. The workers also used the water to clean the knife and their arms and hands, which led to their subsequent contamination. The workers would also frequently use their hands to scoop out water and throw it onto the carcass to clean it from blood and dirt, thereby also contaminating the meat and offal.

The washing of carcasses is forbidden in many countries, including Sweden (European Commission 2004), but it has been described in several slaughterhouses in Sub-Saharan Africa. While the intention is to clean the carcass and organs, it has been shown to constitute a source of bacterial contamination in previous studies in Zambia (Mpundu *et al.* 2019) and Nigeria (Bello *et al.* 2011). Hence, in Paper IV, this practice constituted a severe risk of both occupational exposure and the spread of foodborne disease. However, most of the workers perceived the washing of meat and intestines as crucial to good slaughter hygiene. The workers believed that this procedure made the meat taste good, ensured a good reputation among customers, and prevented the meat from going bad. The workers even believed that consumers could get sick if they ate meat that had not been washed.

At the time of visits, there were no ante-mortem or post-mortem inspections performed by trained personnel. In addition to increasing the risk of the spread of foodborne disease, the absence of ante-mortem inspection services exposes the workers, and potentially also other people present in the slaughterhouse, to a wide array of sick animals (Ray *et al.* 2009; Mohamed *et al.* 2010; Cook *et al.* 2017b). According to the workers, it was rare for the animals to display clinical signs prior to slaughter, although ocular and nasal discharge was sometimes seen. Also, it was reportedly uncommon for the workers to discover macroscopic abnormalities on the carcass or organs after slaughter. The most common findings were ulcers on the intestines, followed by soft, watery lungs, sometimes filled with pus, and occasionally sores on the lungs or in the thoracic cavity. Upon noticing these findings, the worker would inform the customer. According to two of the respondents, buyers could demand a discount or a refund if parts of the meat and/or certain organs needed to be discarded. This could potentially make the workers less inclined to reveal post-mortem findings, as it would reduce their income.

Figure 8a-d. a) A slaughterhouse worker washing hands in a bucket with water mixed with intestinal contents. b) A worker preparing slaughter offal c) Organs kept on the pelt of the slaughtered animal for identification d) Dehiding of a recently slaughtered goat. Note the lollipop in the worker's mouth. Photographs: Sara Lysholm

Table 6: Selected human exposure risks to zoonotic pathogens connected to the slaughterhouse at the Lusaka small livestock market. 'Workers' refers to occupational hazards for the slaughterhouse workers. 'Non-workers' refers to the risks to people in the slaughterhouse who do not perform slaughter procedures, mainly customers. 'Meat and organs' refer to risks of contamination of the carcass and offal.

		Workers	Non-workers	Meat and organs
Infrastructure	-Batch slaughter*	-	-	X
	-Sewage outlet clogging, floors flooded with contaminated water	X	X	X
	-Customers allowed to move freely within the building	-	X	X
	-Customer waiting area only partially demarcated	-	X	X
Sanitation	-Insufficient cleaning of floor and surfaces	X	X	X
	-Poor routines for cleaning tools etc.	X	-	X
	-Washing tools with contaminated water	X	-	X
	-Carcass washing with contaminated water	X	-	X
	-Meat preparation on the same piece of cardboard, throughout the day	X	-	X
	-No usage of soap to clean the knife	X	-	X
Personal hygiene	-Poor access to hand sanitation facilities	X	X	X
	-Handwashing with contaminated water	X	-	X
	-No use of soap to clean hands and arms	X	-	X
	-No work clothes	X	-	X
	-No work footwear	X	-	X
	-No personal protective equipment (gloves, aprons etc.)	X	-	X
Other	-No ante-mortem inspection	X	X	X
	-No post-mortem inspection	X	X	X
	-No formal training of slaughterhouse workers	X	-	X
	-Fly infestation	X	X	X
	-Frequent forming of condensation	X	X	X

*Batch slaughter refers to the practice of performing all steps in the slaughter procedure in one place, next to animals and carcasses at other steps in the procedure

5. Conclusions

This thesis has investigated different aspects of the circulation of transboundary animal diseases (TADs) in small ruminants in farms and markets in Zambia and the Tanzania-Zambia border region. First, it focused on determining the impact of trade and border proximity on the seroprevalence of selected TADs. The second focus was to investigate how the perceptions and practices of small ruminant traders influenced the risk of disease dissemination through trade. Lastly, the thesis aimed to determine the presence of selected zoonotic pathogens at two urban informal small livestock markets, and the possible impacts of this presence on public health in Zambia. The main findings of this thesis are:

- Mccp, FMDV, CCHFV and *Brucella* spp. were circulating in the small ruminant population in different regions in Zambia. While RVFV appeared to be present in the Tanzania-Zambia border region, only limited serological indications of the circulation of PPRV, and no signs of SGPV, were found in this area.

- PPRV, FMDV, RVFV and *Brucella* spp. were circulating in the sheep and goat population in Tanzania close to the Zambian border. However, only limited serological indications of the circulation of SGPV were detected.

- With the possible exception of brucellosis, the impact of proximity to an international border appeared negligible. The impact of trade varied considerably between different TADs.

For example, the purchase of small ruminants from markets or traders was identified as a risk factor for FMDV. While the introduction of new animals to the herd was not identified as a risk factor or protective factor for most of the pathogens, selling sheep and goats frequently (*Brucella* spp.) and not selling at all (FMDV, Mccp) were identified as potential risk factors.

- There were considerable risks of dissemination of disease within the informal small ruminant trade system in Zambia, and this risk was aggravated by traders' perceptions and practices. For example, the traders frequently traded sheep and goats showing clinical signs of disease as they either appeared unaware or indifferent to the associated risks, experienced financial constraints or assigned responsibility for disease prevention to other value chain actors.

- In previous research, lack of knowledge is a common reason given for why traders engage in behaviours that can contribute to the transmission of diseases, such as trading sick animals. In the work included in this thesis, the respondent traders bought and sold sick small ruminants for a range of different reasons. Only some of the respondents could therefore be expected to change their behaviour following a knowledge improvement campaign.

- When the respondent small ruminant traders were asked to describe a model trader, they often mentioned being good at establishing favourable relationships with customers. This indicates that influencing client demands would be likely to be an efficient way of modifying traders' perceptions and practices.

- *Brucella* spp. and *Coxiella burnetii* were circulating in the source population for the Lusaka and Kasumbalesa sheep and goat markets. This can have severe potential impacts on public health in Zambia, since both bacteria are transmissible to humans at slaughter and, in the case of *Brucella* spp., when consuming undercooked meat.

- Several occupational hazards and risks of foodborne disease spread were seen at the informal slaughterhouse at the small livestock market in Lusaka. The washing of intestines in a bucket of water was perceived by most of the slaughterhouse workers to be the most important step to ensure good hygiene at slaughter. However, as this led to faecal contamination of the water in the bucket, which was later used to wash the carcass, the knife and the slaughterer’s arms and hands, in reality this was one of the practices that compromised hygiene the most.

6. Future perspectives

Small ruminants play important roles in livelihood security around the world. Following the projected increase in demand for mutton meat (FAO & OIE 2016), and with climatic projections indicating less suitable crop farming conditions (Ramírez Villegas & Thornton 2015), these species are likely to become more important in future. However, their role is threatened by infectious diseases, which could severely hinder the sustainable intensification of the small ruminant sector (Sherman 2011). Diseases such as PPR are continuously spreading into new areas, with severe implications for local sheep and goat populations and peoples' livelihoods (FAO & OIE 2016). Researching the important mechanisms behind their spread is imperative if the impact of small ruminant diseases is to be limited. This includes understanding the effect of societal structures and networks, such as the organization of trade, trade fluctuations and animal movement patterns (Ayebazibwe *et al.* 2010; Di Nardo *et al.* 2011; Sherman 2011), and how these patterns are altered e.g. close to important infrastructures and larger towns. To understand the human impact on the risk for disease spread, the focus of research also needs to move beyond the individual and beyond curing knowledge gaps. The actions of farmers and traders are not merely an outcome of their knowledge, but also of cultural expectations and structural limitations, for example (Burton 2004; Shortall *et al.* 2017).

Transboundary diseases often attract considerable attention from policymakers, researchers, stakeholders and the public (Rossiter & Al Hammadi 2008). While endemic diseases typically receive less focus (Carslake *et al.* 2011), their impact on animal welfare and farmers' livelihoods can be considerable, and they are often perceived by stakeholders to be more important than transboundary diseases (Rushton *et al.* 2017; Fischer *et al.* 2020a). For research to gain local relevance and be accepted by members of the value chain, endemic diseases should also be included in future research efforts.

Small ruminants have historically often been of lower priority for policy makers, veterinary services and stakeholders compared with e.g. cattle. In many low- and lower-middle-income countries, access to veterinary diagnostic services is limited (Mathew 2017; Chapoto & Subakanya 2019; George *et al.* 2021). This, combined with the fact that poverty-constrained households are often unable to spend scarce resources on diagnostic services, means that infectious diseases in small ruminants often go undetected (de Haan *et al.* 2015). Therefore, it is important to improve knowledge about which pathogens are causing which clinical signs in sheep and goats, and also how different microorganisms together cause disease in these species. Metagenomic studies, i.e. the ability to study uncultured microbial communities in samples without prior sequence knowledge, can provide important insights and holds great future promise (Blomström 2011).

Animal markets play vital socio-cultural roles (Naguib *et al.* 2021). However, these markets have repeatedly attracted attention for their presumed role in the emergence and spread of animal diseases, most recently during the Covid-19 pandemic (Naguib *et al.* 2021; WHO 2021). Following disease outbreaks, bans on market activities are often introduced temporarily (Yuan *et al.* 2015; Xiao *et al.* 2021). By removing the supply of, but not the demand for, market products, these top-down-imposed bans risk pushing the trade into the black markets (Bonwitt *et al.* 2018), and hence may increase rather than decrease the risk of disease spread (Roe & Lee 2021). Therefore, taking measures that reduce the risk of disease emergence and spread at animal markets is important, while allowing market activities to be maintained. Mapping the presence of pathogens at live animal markets, along with key mechanisms for animal-animal and animal-human spread, is essential. Furthermore, when formulating interventions aimed at reducing the risk of disease spread at markets, the inclusive and voluntary participation of the stakeholders concerned is required if the change is to be sustainable (Lysholm *et al.* 2020).

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Popular science summary

Sheep and goats play an important role for poor people in the Global South. They are comparatively cheap to buy and keep, and they can survive under harsh conditions. As such they are important sources of meat, milk and hides, as well as of income and savings. Therefore, when these animals get ill it can have a serious impact on peoples' livelihood. The impact can be particularly large following an outbreak of a transboundary disease. Transboundary diseases are infectious diseases that can spread quickly over large distances, for example between countries and regions. They can cause high rates of disease and death and are therefore a constant threat to peoples' livelihoods and society. Some transboundary diseases are also zoonotic, which means that they can infect and cause disease in both humans and animals.

In order to minimise the occurrence of transboundary diseases, it is important to identify the areas in which they are present. It is equally essential to identify the mechanisms that contribute to their spread. This thesis focused on determining the presence of selected transboundary diseases in blood samples collected from sheep and goats. The impacts of potential risk factors were also studied, focusing on the effects of trade and proximity to international borders. In addition, we investigated how traders and slaughterhouse workers understand and act on diseases in sheep and goats, and how this can lead to increased or decreased risks of disease spread. Lastly, we studied the presence of zoonotic diseases at two sheep and goat markets in Zambia, and the potential impact of this presence on human health.

In the first paper, we investigated the presence of the transboundary diseases peste des petits ruminants (PPR), foot and mouth disease (FMD), sheeppox and goatpox (SGP), Rift Valley fever (RVF) and brucellosis in sheep and goats in the Tanzania-Zambia border region. The paper also analysed the impact of potential risk factors, focusing on trade and proximity to an international border. The results indicated that most of the above-mentioned diseases are present. However, whether SGP occurs in the region or not, and whether PPR is present in Zambia, needs to be investigated in future studies. Buying sheep and goats from traders or at markets was associated with a greater risk to being exposed to FMD and in general, herds situated close to an international border were less likely to have been exposed to the surveyed diseases.

The second paper aimed to further investigate the presence of selected transboundary diseases in goats in Zambia – contagious caprine pleuropneumonia (CCPP), FMD, RVF, Crimean-Congo haemorrhagic fever (CCHF) and brucellosis. Also, the impact of potential risk factors was studied, focusing on trade and border proximity. The results indicated that most of the surveyed diseases were present. As only two goats tested positive for RVF, the presence of this disease should be further investigated in future studies. The impact on trade varied depending on the disease, and generally herds in districts adjacent to an international border were less likely to have been exposed to the surveyed diseases.

Trade in livestock can cause spread of infectious diseases, and the trader plays a key role in the trade process. In the third paper, interviews with small ruminant traders were performed at the two largest informal markets for small livestock in Zambia. The goal was to find out how small ruminant traders understand and act on diseases in small ruminants in trade. It was found that many traders believed that it was acceptable to sell sheep and goats that were showing signs of sickness, at least under certain circumstances. Poor knowledge did not appear to be the only reason for this, which is in contrast to the findings of several previous studies. When asked to describe a ‘good trader’, many emphasised taking good care of customers. This indicates that traders’ behaviours are influenced by customer demands.

Live animal markets bring humans and animals of different species into close proximity. Humans attending these markets and associated slaughterhouses risk being infected by a zoonotic disease, for example when slaughtering an infected animal or when consuming animal-derived food. In the fourth paper, the presence of the zoonotic diseases brucellosis, Q-fever/coxiellosis and RVF was analysed in sheep and goats present at the same small ruminant markets as those studied in the third paper. The results indicated that both brucellosis and Q-fever are circulating in the markets' source populations. The activities at a market slaughterhouse were also observed, and interviews and group discussions were held with slaughterhouse workers. Multiple slaughter procedures were observed that could expose slaughterhouse workers to zoonotic disease and lead to the contamination of meat and organs. Hence the study shows that the informal small ruminant market system in Zambia poses risks to human health.

In conclusion, the results in this thesis indicate that several transboundary animal diseases are circulating in the sheep and goat populations in Zambia and in the Tanzania-Zambia border region. Human activities at small livestock markets in Zambia can contribute to their spread, both to other sheep and goats and to humans. By taking steps to reduce the presence of sheep and goat infectious diseases, considerable improvements can be made to the health and welfare of animals and humans alike.

Populärvetenskaplig sammanfattning

Får och getter fyller viktiga funktioner för människor i globala södern. De är förhållandevis billiga att köpa och äga, och de kan klara sig under magra levnadsförhållanden. De utgör viktiga källor för bland annat inkomst, kött, mjölk och päls. Sjukdomar hos får och getter kan därför få allvarliga konsekvenser för människors levnadssituation. Effekterna är förmodligen extra påtagliga när de orsakas av så kallade ”transboundary animal diseases”, vilket kan översättas till ”gränsöverskridande djursjukdomar”. Dessa infektionssjukdomar har en förmåga att spridas snabbt över stora avstånd, till exempel mellan olika länder och regioner. De kan orsaka stora antal sjukdomsfall och dödsfall, och utgör därför ständiga hot mot människors liv och hälsa och mot samhället. Vissa av sjukdomarna är zoonotiska, vilket betyder att de kan infektera och orsaka sjukdom hos såväl djur som människor.

För att minska förekomsten av dessa gränsöverskridande djursjukdomar är det viktigt att hitta de områden där smittorna förekommer. Det är också viktigt att identifiera mekanismer som bidrar till deras spridning. I denna avhandling undersöktes förekomsten av ett fåtal utvalda gränsöverskridande sjukdomar i blodprover från får och getter. Påverkan av vissa potentiella riskfaktorer studerades också, med ett fokus på inverkan av handel och närhet till internationella gränser. Vi har också undersökt hur zambiska djurhandlare och slaktare resonerar och agerar när får och getter visar tecken på sjukdom, och hur detta kan leda till minskad eller ökad risk för sjukdomsspridning. Slutligen har vi studerat förekomsten av utvalda zoonotiska sjukdomar på får- och getmarknader i Zambia och vilka risker detta kan få på människors hälsa.

I den första studien undersöktes förekomsten av de gränsöverskridande sjukdomarna peste des petits ruminants (PPR), mul- och klövsjuka (FMD), får- och getkoppor (SGP), Rift Valley feber (RVF) och brucellos hos får och getter i gränsregionen mellan Zambia och Tanzania. Påverkan av potentiella riskfaktorer analyserades också, med fokus på inverkan av gränsnärhet och djurhandel. Resultatet tyder på att de flesta av de studerade sjukdomarna förekommer i regionen. Huruvida SGP finns i gränsområdet, och om PPR förekommer i Zambia eller inte, behöver dock utredas vidare i framtida studier. Att köpa får och getter på marknader eller av djurhandlare var associerat med ökad exponeringsrisk för FMD. Flockar som fanns i närheten av internationella gränser löpte generellt lägre risk att vara exponerade för de studerade sjukdomarna.

Den andra studien ämnade att ytterligare undersöka förekomsten av gränsöverskridande sjukdomar hos getter i Zambia. Sjukdomarna smittsam pleuropneumoni (CCPP), FMD, RVF, Krim-Kongo blödarfeber och brucellos, inkluderades i studien. Utöver detta analyserades också påverkan av olika riskfaktorer, med fokus på djurhandel och gränsnärhet. Resultaten pekar på att de flesta av sjukdomarna förekommer i de undersökta områdena. Eftersom bara två getter testade positivt för RVF bör förekomsten av denna sjukdom utredas vidare i framtida studier. Inverkan av handel varierade mellan de olika sjukdomarna, och generellt var risken för exponering lägre bland flockar i gränsnära distrikt. För brucellos utgjorde dock gränsnära distrikt en riskfaktor.

Handel med djur kan bidra till spridning av infektionssjukdomar, och i denna process spelar djurhandlaren en viktig roll. I den tredje studien utfördes intervjuer med djurhandlare på de två största informella marknaderna för får och getter i Zambia. Målet var att förstå hur dessa djurhandlare resonerar och agerar avseende sjukdom hos får och getter i handel. Vi upptäckte att många djurhandlare ansåg att det var okej att sälja djur med tecken på sjukdom, i alla fall under vissa förutsättningar. Till skillnad från tidigare forskning tyder resultaten i denna studie på att detta inte enbart beror på bristfällig kunskap. När djurhandlarna ombads att berätta vad som kännetecknar en bra djurhandlare betonade många vikten av att ta väl hand om sina kunder. Detta tyder på att djurhandlarnas beteende kan påverkas av kundernas efterfrågan.

Människor och djur av olika arter kan komma i nära kontakt med varandra på djurmarknader. Människor som vistas på dessa marknader och på de slakterier som ofta finns på marknadsområdena, riskerar att smittas av zoonotiska sjukdomar. Detta kan till exempel ske vid slakt av ett infekterat djur eller vid konsumtion av exempelvis kött. I den fjärde studien undersöktes förekomsten av de zoonotiska sjukdomarna brucellos, Q-feber/coxiellos och Rift Valley feber, hos får och getter på samma djurmarknader som studerades i den tredje studien. Resultatet tyder på att både brucellos och Q-feber cirkulerar i de flockar vars djur senare säljs till marknaderna. Dessutom observerades de dagliga aktiviteterna på ett marknadsslakteri, och intervjuer och gruppdiskussioner genomfördes med slaktarna. Flera slaktprocedurer observerades som både kan exponera slaktarna för zoonotiska sjukdomar och leda till kontaminering av kött och organ. Studien visar därmed att det informella marknadssystemet utgör ett potentiellt hot mot människors hälsa.

Sammanfattningsvis tyder resultaten i denna avhandling på att flera gränsöverskridande sjukdomar cirkulerar i Zambias och Tanzanias får- och getpopulationer. Människors aktiviteter på zambiska får- och getmarknader kan bidra till spridning av dessa sjukdomar, såväl till djur som till människor. Genom att vidta åtgärder för att minska förekomsten av dessa sjukdomar kan stora förbättringar åstadkommas för både djurs och människors hälsa och välbefinnande.

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III

PLOS ONE

RESEARCH ARTICLE

Perceptions and practices among Zambian sheep and goat traders concerning small ruminant health and disease

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Abstract

Trade in animals and animal products is a key factor in the transmission of infectious diseases. Livestock traders play an important role in this process, yet there is little knowledge of traders' perceptions of animal disease or their associated actions. The aim of this study was to investigate perceptions and practices of Zambian small ruminant traders with regard to sheep and goat health and disease. It also analysed how existing perceptions and practices might affect risks of disease transmission through trade. A case study was performed at the two largest small livestock markets in Zambia: the Lusaka market in the capital and the Kasumbalesa market near the border with the Democratic Republic of Congo. Semi-structured interviews with 47 traders performed in April-May and September 2018 represent the core material. Zambian small ruminant traders frequently trade animals that have clinical signs of disease, either because they appear unaware or indifferent to the associated risks, experience financial constraints or assign responsibility for disease prevention to other value chain actors. In their decision about whether or not to sell a visibly sick small ruminant, traders appear to consider whether the clinical sign is perceived as 'natural' or the result of an illness, whether the buyer is aware of the animal's health condition, and whether the animal is sold for consumption or breeding purposes. Traders appear to regard the veterinary certificate required to transport small ruminants in Zambia as proof of health, placing the responsibility for potential disease in traded animals on the veterinary authorities. In their description of a model trader, taking good care of and being sensitive to customer needs was emphasized, indicating that an efficient way to encourage traders to change their behaviour is to influence customer demands. In contrast to the focus in previous studies on identifying and filling knowledge gaps, the present study show that lack of knowledge is not central to why traders engage in disease-transmitting behaviour. Greater awareness of other reasons for certain perceptions and practices could lead to the formulation of risk communication strategies and mitigation measures that are relevant for the local context, as well as alternative strategies for changing trader behaviour.

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Introduction

Market participation and trade among smallholder livestock farmers has received increased recognition in recent years as it is expected to offer an important route out of poverty [1, 2]. However, with growing livestock trade and market engagement, the risk of disseminating infectious diseases also increases. Livestock markets serve as congregation points for animals from various regions, where pathogens can be disseminated, e.g. highly pathogenic avian influenza (HPAI) in multiple locations [3], sleeping sickness (African trypanosomiasis) in Uganda [4] and *peste des petits ruminants* across Africa [5–8]. A significant proportion of the animals sold at urban markets are slaughtered for human consumption, which in the case of substandard or absent veterinary inspections and unhygienic slaughter procedures constitute severe public health risks [9]. Outbreaks of human disease have also been linked to visiting animal markets, e.g. Q-fever [10], SARS [11] and more recently, COVID-19 in Wuhan, China [12].

While much informal livestock trade, especially in rural areas, occurs directly between farmers and customers, it also frequently involves middlemen (traders) who purchase animals from farmers and resell to final customers. Owing to their key position between the producer and the customer, traders can play a central role in the spread or containment of infectious pathogens. Therefore, it is highly relevant for efficient disease control to understand how individuals in this group reason and act in relation to animal health and disease. In rural Zambia, small ruminant trade mostly occurs directly between farmers and customers, but a considerable amount also occurs through traders selling at informal urban markets [9, 13]. The focus of this study was on traders selling animals at these urban markets. Animals that are traded there are often transported over long distances and across several contact points, where sheep and goats from different areas can mix and pathogens be exchanged. Additionally, due to the informal nature of this market chain, market biosecurity and public health protective measures tend to be less controlled by Zambian authorities than in more formalised systems [9]. The health status of small ruminants in Zambia is generally considered good, while several sheep and goat infections with high morbidity and mortality rates are known to be present in neighbouring countries. These include contagious caprine pleuropneumonia (CCPP) [14–17], sheep and goat pox (SGP) [18], and *peste des petits ruminants* (PPR) [7, 14, 16–20]. There is a non-negligible risk of these pathogens being introduced in Zambia, e.g. through informal international trade.

Previous research on the perceptions and practices of farmers with regard to animal disease

The importance of investigating farmers' perceptions and practices concerning animal health and disease in order to find effective ways of containing infections, has recently received increased recognition. It is commonly stated within the field of veterinary medicine that farmers fail to comply with recommendations on e.g. disease control owing to a lack of knowledge. Therefore, the focus is frequently on educating farmers about animal diseases [21]. While improving knowledge is important, studies on farmers in different parts of the world indicate that financial and other structural constraints [22], as well as individual farmers' normative frames of reference [23], sense of responsibility for preventing disease [24], attitudes regarding disease risks [24], and perceived effectiveness of control measures [23, 24] have a greater influence on how farmers act on the basis of disease, treatment and veterinary advice than their factual knowledge.

In recent research, the importance of understanding the concept of "model peers" has been recognised. For example, ideals of what a "good" farmer is have been shown to have a substantial effect on farmers' perceptions and practices. It is acknowledged that since farmers, like people in general, gain social standing by abiding by norms embedded in their community culture [25–27], it is relevant to study this aspect in order to understand why farmers reason and act the way they do. Studies on livestock farmers in Europe and the US have shown that while there is an emphasis on running a financially successful farm [26] and e.g. low mortality rates [24], it is often judged as equally important to have close emotional ties with one's animals and be skilled at judging animal health by eye [22, 28, 29]. "Good farmer" ideals are often locally specific, as geographic closeness, regional conditions and local farming practices have been shown to influence the attributes considered desirable [25].

Previous research on trader perceptions and practices related to animal disease

Results from studies on farmers' perceptions and practices on animal health and disease should not be uncritically extrapolated to traders, since they generally work in different contexts and have different relationships with the animals. For example, traders typically keep each animal for a limited time and so are unlikely to learn about the specific traits of each animal, which has been highlighted by farmers as an important means of identifying and interpreting animal sickness and health [22, 28, 29]. Furthermore, keeping animals during a limited time is likely to reduce incentives to ensure good long-term animal health. To date, only a limited number of studies have been performed with the purpose of understanding the knowledge, attitudes and practices of traders related to animal health and disease. The majority of these are in the form of quantitative questionnaire studies and cover poultry trade and avian influenza (AI) [30–32] or pig trade and African or classical swine fever (ASF and CSF) [33, 34]. Many of these studies focus on determining factual knowledge levels. A common conclusion drawn is that knowledge among traders is low on various disease features, e.g. clinical signs and transmission routes. Therefore, a collective recommendation is often to take action to improve traders' knowledge.

A group of studies stand out for adopting a qualitative approach to the study of traders' perceptions and practices with regard to ASF [35–38]. These studies indicate that traders generally are knowledgeable about major clinical signs and transmission routes [35, 36] and are often aware of their potential role in disseminating ASF. In spite of this awareness, traders frequently engage in activities that can contribute to dissemination of the virus [35–37]. These studies therefore indicate that the risky behaviours employed by traders are unlikely to be due solely to a lack of factual knowledge.

In summary, few studies to date have investigated the relationship between traders' perceptions and practices on animal health and disease and effects on disease transmission. In light of this research gap, the aim of the present study was to investigate perceptions and practices of Zambian small ruminant traders in relation to sheep and goat health and disease, and to analyse how these might affect risks of disease transmission through trade. To meet these aims, the following research questions guided the work:

1. how do Zambian sheep and goat traders define health and disease in their animals;
2. what factors in the wider trading situation are important for determining traders' actions with regard to animal disease;
3. what actions do different disease signs warrant from the trader.

Materials and methods

Description of the case study

The study was designed as a case study, i.e. a study of "a unit of human activity embedded in the real world; which can only be understood in its context" [39]. The case study consists of the two largest small livestock markets in Zambia: the Lusaka small livestock market, situated in the outskirts of Lusaka in Chibolya township, and the Kasumbalesa small livestock market in the Copperbelt province, adjacent to the border point with the Democratic Republic of Congo (DRC). At the time of the visits, both markets were run by the Small Livestock Association of Zambia (SLAZ), a non-governmental association whose primary purpose is to establish a more organised market system for small livestock in Zambia. The Lusaka small livestock market predominantly trades in goats, pigs, chickens, other fowl and sheep. The market place contains animal pens, a veterinary shop and two slaughterhouses–one for small ruminants and one for pigs. At Kasumbalesa small livestock market, goats, pigs, sheep, chicken and other fowl are sold. There was no veterinary shop or designated slaughterhouse for small ruminants at the time of our visits. Many of the respondents conducted trade at both the Kasumbalesa and Lusaka markets, warranting the treatment of trade at these two markets as one case. In both market places, goats were significantly more common than sheep. According to the respondents, trade is highly seasonal, with more market activity around celebrations and festivities, as well as prior to the due date of school fees.

Data collection

Data were collected by the first author at the Lusaka small livestock market in April, May and September 2018, and one field visit to Kasumbalesa in September 2018. Authors 2–4 also visited the Lusaka small livestock market in April 2018 and November 2019, allowing the authors to discuss the material together as a group. In all, 21 days were spent at the Lusaka market and four days at the Kasumbalesa market. All data collection occurred in seasons of low trade activity. The main method of data collection was semi-structured interviews with traders present at the market places; 35 traders in Lusaka and 12 traders in Kasumbalesa. A topic guide developed from the research questions was utilised to navigate the interviews with each respondent, while at the same time keeping an openness to new and unexpected information given by the interviewee [39].

During each visit to Lusaka small livestock market, the majority of traders that had not already participated were approached to obtain oral consent for an interview. Around 10% declined to participate, often due to lack of time. Visits at Lusaka market were repeated until saturation in the information coming out from interviews with traders was reached, meaning that no new variations to answers emerged. At Kasumbalesa market, only four visits were made due to time constraints. As the majority of the traders at both markets were men, less than 15% of the respondents in this study are women. All interviews were performed with an interpreter and responses were noted down by hand by the first author. In Lusaka, most of the interviews were performed in the local language Nyanja, while in Kasumbalesa, Bemba was most commonly spoken. In a small number of interviews, the interpreter and the respondent did not speak the same language, and on these occasions other people at the markets interpreted. These interviews were judged to be of lower quality because they were performed with an untrained interpreter, which later was taken into account in the analysis.

To enrich our understanding of the market and trade situation, and to triangulate the responses from traders, interviews with other value chain members were also performed. In Lusaka, five slaughterhouse workers, four transporters, four veterinary shop workers, three SLAZ employees and 20 market customers (of whom two came from the DRC, two were farmers buying for breeding purposes, and 16 were buying for consumption) were interviewed about their reason for visiting the market, their role in the value chain and their perceptions on animal health and disease. At the Kasumbalesa small livestock market, nine market customers, of whom eight were from the DRC and one a farmer from a nearby town, were interviewed. Only individuals aged 18 and above were allowed to participate. To triangulate the information given during interviews [40], and to get a richer understanding of the trading context, observations were made of all activities at the markets. These observations were noted down by hand by the first author in detailed field notes [41].

Data analysis

Analysis was facilitated by the use of NVivo 12.2.0 software (QSR International, Warrington, UK) and performed by the first author guided by the fourth author. All interviews were thematically coded [42], a process that was guided by the research questions, while being open to new emerging themes. Coding was an iterative process where more detailed themes could be found in the initial broad themes of the research questions, through repeated readings of the material [42, 43] and by theoretically informing later rounds of analysis [43]. For example, early thematic analysis was further refined in the light of the literature on being a 'good farmer'. This led us to identify a sub-theme in the trader-responses, regarding how traders valued being a good trader. Interviews from other stakeholders and observations of market activities were used to enrich and to triangulate the understanding of emerging themes in the trader-interviews. The quotations in the text are based on written notes from interviews and are thus not verbatim, however the meaning and essence of the respondents' words, as supplied by the interpreter, have not been altered.

Ethical considerations

Before starting the work, author 1 and 2 visited SLAZ representatives at the markets to explain the purpose of the study and obtain permission to conduct the research. Prior to commencing the interviews, oral informed consent was obtained from every participant. Care was taken to ensure respondent anonymity and confidentiality by only collecting personal data relevant for the study and never disclosing information related to individual informants to members outside the research team. The study sought and received ethical approval by the ILRI Institutional Research Ethics Committee (ILRI-IREC2018-04).

Results

The traders in this study came from both rural and urban areas. Traders from rural areas are commonly farmers in addition to their trading activities and will often trade both their own animals and those purchased from other farmers. The area that different rural traders cover in search of sheep and goats for sale varies, but can extend to up to a few hours on foot, by bike or motorized vehicle. Previously bought small ruminants often accompany the traders from one village to the next and are allowed to intermingle with the animals there. Several traders also keep the sheep and goats bought for trade with their own animals at home for some time. Due to high transportation costs, rural traders generally co-organise transport to the markets, resulting in more intermingling.

Prior to transporting animals to markets, a stock movement permit has to be obtained. In brief, this process includes obtaining a certificate from the village headman and the police, who mainly certify that the animals have not been stolen. Furthermore, a certificate from the district council is required, as well as a veterinary clearance to certify that the animals are healthy [44, 45]. The permit, and ideally also the health of the transported animals, is then controlled at police and veterinary checkpoints along the larger roads [38]. Upon arriving at the Lusaka market, the rural traders generally sell their animals to urban traders, who then sell to market customers. More rarely, rural traders sell animals directly to markets customers.

The majority of the sheep and goats sold at the Lusaka and Kasumbalesa small livestock markets are slaughtered for human consumption. In Lusaka, many are slaughtered at the local slaughterhouse, while others are taken to nearby slaughterhouses or slaughtered by the buyers themselves. At the Lusaka slaughterhouse, no ante- or post-mortem inspection was performed at the time of the study visits. Customers who frequently purchase small ruminants for consumption include suppliers for restaurants, bars, hotels, market meat sellers, butchers and people buying for home consumption. A small fraction of purchased animals is kept alive and transported back to farms, mainly for breeding purposes. At both markets, but particularly in Kasumbalesa, traders from neighbouring countries (mainly DRC), purchase small ruminants and transport them back to their home countries where they typically are slaughtered for consumption.

Traders' perceptions of small ruminant health and disease

When the respondents were asked what they looked for when purchasing sheep and goats, nearly all of them answered that they primarily look at the size of the animal and whether or not it appears healthy. Many defined being healthy as the absence of various clinical signs, e.g. dull coat, weakness, nasal discharge and diarrhoea. However, the majority mentioned fatness as the most important indication of health, and some respondents even believed that it is impossible for a fat sheep or goat to be ill.

> "*I only buy fat and healthy goats, they don't get sick.*"
>
> Trader at the Lusaka small livestock market

Several respondents stated that it is rare to witness signs of disease in small ruminants at the market. However, when asked specifically about whether they had witnessed specific clinical signs, e.g. diarrhoea or coughing, several traders admitted that they observed such signs on a regular basis. The clinical signs that were described included diarrhoea, coughing, ocular and nasal discharge, abortion, sudden death and "skin disease", i.e. an ectoparasitic skin infection manifested by severe pruritus and thickened abraded skin. When asked which specific diseases the respondents had experienced in traded sheep and goats, they generally described clinical signs rather than specific diseases. The only exception was foot and mouth disease (FMD), which was mentioned by some respondents. When asked about causes of the clinical signs, no respondent spontaneously mentioned the possibility of an infectious pathogen. Only when asked specifically whether certain clinical signs could be caused by viruses, bacteria or parasites did a few respondents answer that this was a possibility. Instead, the respondents generally believed that abortion, for example, is invariably caused by beatings or being squeezed together in transport, diarrhoea by feed changes or consuming unsuitable objects, coughing and ocular and nasal discharge by weather changes and dirty environments, skin disease by rain and insufficient cleaning of the pen, and sudden death by stress during transport.

> "*With my own goats at home I have problems with skin disease, which is caused by rain. I treat it by building a shelter for them. I don't understand why they get it! It can't just be because of the rain; rainwater in itself is not harmful. There must be something else as well, in addition to the rain.*"
>
> Trader at the Lusaka small livestock market

At the same time, most respondents were aware that diarrhoea, coughing and skin disease, for example, can be transmitted between animals. Commonly mentioned transmission pathways included airborne spread, eating or drinking together, and coming into contact with animals from far away, e.g. through trade.

"*Trade can contribute to the spread of diseases. For example, if you buy a goat with diarrhoea and take it home, and then the diarrhoea ends up on the grass which another goat eats, it can also get diarrhoea.*"

Trader at the Lusaka small livestock market

Trade in small ruminants showing signs of clinical disease

Several respondents stated that they would not engage in trading sick small ruminants. Some attributed this to their own experience and ability to detect diseased sheep and goats, which prevented them from buying and hence also selling sick animals. Other respondents had a humbler view of their ability to identify disease in small ruminants, but believed that they did not keep the animals long enough for them to develop disease. However, the most commonly cited reason for animals not being sick in the care of the traders was the requirement to obtain veterinary clearance as part of the stock movement permit prior to transporting the animals to markets. Many regarded this process as a guarantee of health and the absence of disease in all animals that were being traded at the markets.

"*It would be possible for trade to contribute to spreading animal diseases, but not with the stock movement permit system. The permit ensures that only healthy animals are traded.*"

Trader at the Lusaka small livestock market

Some respondents argued that the system with stock movement permits means that excluding sick animals from trade is the responsibility of the veterinary authorities, not the members of the value chain.

"*It is the responsibility of the council and the veterinarians to prevent diseases from getting to Chibolya.*"

Trader at the Lusaka small livestock market

However, the interviews indicated that the process for acquiring a veterinary clearance varied considerably between districts. According to a small number of informants, the veterinarian in their district just signs the certificate without seeing the animals. Others stated that the veterinarian performs a visual examination of all the animals as well as a physical examination of a selected few. The most common answer was that the veterinarian performs a visual examination of the whole group (not examining individuals) and then issues the certificate. In addition, at veterinary checkpoints during transportation, several traders reported that while the permit itself is controlled, the animals themselves are only looked at occasionally. Also, according to several informants, as corruption is widespread in Zambia, bribing personnel at checkpoints is common, which further impedes the efficiency of the stock movement permit system.

Several interviewees initially stated that they never had and never would engage in trade with animals that were sick. Many stressed that it could be harmful for their business as they risk losing customers if it was detected and exposed, or that they could get in trouble with the Zambian authorities. Others described the sale of sick animals as immoral and that it conflicted with their religious beliefs, while others refrained because it "felt wrong" or because of a reluctance to contribute to disseminating disease among sheep and goats or to consumers.

> "*I would never sell a sick goat, no matter how mild the disease was. If I did, I would contribute to spreading the disease or putting people at risk of getting sick.*"
>
> Trader at the Lusaka small livestock market

However, with several respondents, a different picture emerged when enquiries were made about whether it is acceptable to sell sheep and goats with specific clinical signs. The majority of the informants believed that selling sheep and goats that are coughing and have nasal and ocular discharge is acceptable, since they either perceived these clinical signs to be very mild or to not stem from disease, but rather from a dirty environment or the weather. According to some, trading sheep and goats with coughs or ocular and nasal discharge is acceptable, but only when it is not caused by disease. When asked to explain how they could be sure whether a clinical sign was due to a disease or not, some admitted that they could not be sure, while others stated that they examined the general health appearance of the small ruminant in question. More specifically, if a coughing animal otherwise appears healthy (i.e. it is not thin or weak for example), they would deduce that it is fit for trade. However, reasoning varied significantly concerning the trade of sheep and goats with diarrhoea and what traders referred to as skin disease. Some believed that it is acceptable since they perceived these clinical signs to be mild and easily treatable, and in the case of diarrhoea, that it is not caused by disease but rather by ingesting unfamiliar feeds or unsuitable objects. Others were of the opinion that selling sheep and goats with diarrhoea or an ectoparasitic skin infection is deeply immoral because they are severe, sometimes deadly diseases and are often difficult to treat.

Upon probing, it turned out that several interviewees were prepared to sell sick sheep and goats with any clinical signs of illness. Some participants considered selling sick animals to be an acceptable practice, provided the buyer is made aware of the health status of the small ruminant. Additionally, a few respondents were comfortable with trading diseased animals provided they are sold for consumption and not for keeping by farmers, as they believed that the disease cannot be transmitted to humans, only to other sheep and goats. Two traders stated that since customers at the markets did not care about whether the animals they purchased were sick or not, they were also choosing to overlook it.

> "*Kasumbalesa is the end point for the goats and the sheep. The majority are being sold for consumption and then it does not matter, nobody cares whether they are sick or not.*"
>
> Trader at the Kasumbalesa small livestock market

However, several respondents expressed feelings of moral conflict, but did not see any other financially feasible alternative than selling the animal. The possible negative financial impacts mentioned by the traders included lost income for the diseased small ruminant as well as costs associated with transporting the sick animal away from the market.

> "*I know selling sick animals is wrong, but what can I do? I don't have the ability to treat them like I would if my goats at home got sick. I can't transport them back home because it's too expensive. My only option is to sell them to avoid losing money.*"
>
> Trader at the Kasumbalesa small livestock market

A few respondents stated that they would attempt to sell the animal quickly after discovering signs of disease to avoid it succumbing to illness prior to being sold. One trader disclosed that he would slaughter the sick animal and sell the meat to avoid lost income, since he would get paid more for the meat than for an animal displaying clinical signs of illness.

The majority of respondent traders reported avoiding purchasing sick sheep or goats. This was mainly out of fear of financial losses, either if the sick animal died or if it transmitted the disease to the other sheep and goats. Nonetheless, some interviewees mentioned that they sometimes buy small ruminants with diseases that they perceive to be mild and/or treatable at a low cost, such as ocular and nasal discharge or skin disease. They then treat the illness and make a profit by selling the animal at a usual price.

When small ruminants with signs of disease appear at the market, perceptions regarding the correct mode of action varied significantly. None of the traders reported the disease to market officials or authorities. A few informants stated that they would keep the sick animal for various lengths of time to allow it to recover. Some mentioned buying medicines, but this was uncommon. The most common action mentioned was to proceed with the sale of the sick animal without attempting treatment or other further actions. During the study visit to the Lusaka small livestock market, no preventative measures were instituted for the market as a whole, but according to SLAZ personnel, there were plans to introduce such measures, primarily in the form of training workshops for value chain members. When individual traders were asked whether they took disease prevention measures at the market, the majority replied that they did not, although some mentioned providing feed and water of good quality for their animals. In spite of this, all the informants took at least one disease prevention measure, most commonly cleaning the pen. However, this was not necessarily regarded as a preventive measure, but was used to attract customers.

Traders' perceptions of public health-related hazards and the practices that cause them

Several interviewees were aware of the hazards associated with the consumption of sick animals, primarily the risk of falling ill from the same disease, but many also feared residues from previous medical treatments. However, several respondents would consume meat from sick animals, despite being aware or at least suspicious of the risks associated with this.

> "*Every cold season my village experiences a strange outbreak that affects all grazing animals. They suddenly collapse and die, blood comes out of their noses and their bodies become bloated. When we open the carcasses, their lungs are filled with blood. We haven't asked the veterinarians for help because they are located very far away from us. We usually eat the carcasses to avoid losses.*"
>
> Trader at the Lusaka small livestock market, describing the consumption of possible anthrax cases

Some participants explained their readiness to consume products from sick animals by not perceiving various clinical signs as the result of disease, but rather due to what is perceived as natural processes. Others expressed confidence in their own ability to determine whether the clinical sign poses a health risk to the consumer or not. A few respondents described a strong love for eating meat and an unwillingness to waste food.

> "*Have we ever slaughtered an animal because it was sick? Yes, many times, we ate the meat and nothing happened! It's because we love meat so much. We'd rather take the risk of getting sick than waste food.*"
>
> Trader at the Lusaka small livestock market

Furthermore, several participants believed that they have taken protective measures that remove the risk of food-borne infections, for example by always boiling meat before consumption or discarding certain organs (e.g. the intestines if the animal has diarrhoea, lungs if it is coughing etc).

A practice that perhaps poses greater risks to public health than slaughtering and consuming diseased sheep and goats is consuming animals that have died on their own. Most respondents stated that although they knew several people who ate animals that had died on their own, they would never do this themselves, either due to a strong feeling of repulsion, religious beliefs or out of fear of succumbing to the same illness as the animal. Respondents who admitted to consuming self-dead animals often justified this by their great love of meat and unwillingness to waste food. Additionally, several misconceptions were identified among the respondents, e.g. that boiling the meat ensures that disease cannot be spread to the consumer.

"*Eating the meat from a sick animal or from an animal that has died on its own is okay as long as the meat is boiled first.*"

Trader at the Lusaka small livestock market

At the Lusaka and Kasumbalesa markets, there were designated locations for disposing of dead animals, and thus the sale of self-dead animals was not an accepted practice. Most of the traders interviewed stated that they had never and would never sell the body of an animal that had died on its own, either because it is immoral or forbidden according to their religion. However, a few traders admitted to selling bodies of animals that had died on their own, and several respondents stated that some buyers visit the markets solely to buy dead animals at cheap prices. One transporter described how he would sell animals that had died during transport, most commonly to meat vendors outside bars that specialised in selling meat to people on their way home after a night out.

"*I sometimes sell the bodies of animals that die during transport for a cheap price. I do it because I feel bad for the owner who needs the income from the sale. Usually they are sold to owners of barbeque stands outside bars.*"

Transporter and trader at the Lusaka small livestock market

Being a "good trader"

When the respondents were asked about being a "good trader", it was clear that being perceived as good by their peers was considered important. The most commonly mentioned characteristic of a good trader was being good at dealing with people, more specifically at establishing favourable relationships with customers, even if they are rude, and being a skilled price negotiator. Many also emphasised the importance of keeping the trade environment clean because this attracts customers.

"*A model trader is someone who can relate to customers and be good to them, even if they are rude back. A good trader will also keep the environment clean to attract customers*"

Trader at the Lusaka small livestock market

Some also defined a "good trader" as someone who keeps animals that look healthy. However, this was mainly emphasised because it attracts market customers rather than because it is beneficial from an animal health perspective.

"*A good trader is someone who trades with animals that are clean and look fat and healthy, since this is what attracts customers*"

Trader at the Lusaka small livestock market

Discussion

The Lusaka and Kasumbalesa small livestock markets congregate small ruminants from different regions across Zambia and therefore pose major risks for disease dissemination. Furthermore, as the majority of animals sold enter the human food chain, many of which are slaughtered without ante- or post-mortem inspections, the Zambian small ruminant trade system poses a significant public health risk. The results of this study show that the risks of disease dissemination and to public health are increased by traders' practices. The majority of the respondent Zambian sheep and goat traders were found to be either engaged in trade with diseased small ruminants or believed that it is more or less acceptable to do so, at least under certain circumstances. Several traders restricted themselves to only engaging in trade with sick sheep and goats when the clinical sign is considered mild or due to natural processes, or to only selling diseased small ruminants for consumption and not to farmers. Many interviewees reasoned that the Zambian stock movement permit system absolves them from responsibility for preventing disease dissemination, since the animals have been certified as healthy by a veterinarian and are subsequently checked at veterinary checkpoints enroute to the markets. However, this study indicates that the stock movement permit system is not adequately implemented to detect disease, and previous research has demonstrated that the veterinary checkpoints are often undermanned and commonly bypassed [38]. It appears as though the transfer permit system has primarily made value chain members focus on obtaining the correct paperwork, with limited reflection on why this is important, rather than actively taking steps to prevent the dissemination of disease.

The respondents' motivation for participating in trade with sick small ruminants can be grouped into three different categories. The first category expressed feelings of moral conflict, but lacked the financial means to withhold the animals from sale or transport them away from the market. The second category were either unaware of the associated hazards or appeared unwilling to accept them as such, e.g. by (strategically or otherwise) downplaying the relevance of certain clinical signs or avoiding to classify them as signs of disease. The third category attempted to assign the responsibility elsewhere, either to buyers by making them aware of the animal's health condition or to the authorities who are responsible for issuing the pre-transportation veterinary certificate. In previous research among traders, lack of knowledge is a commonly stated reason for risky behaviours such as trading sick animals [30–32, 34]. The traders in the current study trade diseased small ruminants for a span of different reasons and would therefore be likely to need different forms of intervention strategies to stop this risky behaviour. The results of this study indicate that only some traders would be significantly affected by a knowledge improvement campaign. Consequently, there is a clear need for research and outreach projects to adapt their agendas and move beyond simply attempting to improve knowledge among value chain actors.

While research on traders' perceptions and practices related to animal health and disease is limited, a significant number of studies have been performed with the same focus on livestock farmers. Similar to studies focusing on farmers' perceptions of animal welfare and disease, the present study indicates that the wider culture of trading and being a respectable trader in the eyes of one's peers is an important driver for how traders reasoned and behaved in relation to sick animals. However, this study clearly shows that the traders relate to a different culture and context when creating their perceptions on animal health and disease than the farmers in studies on the cultural shaping of farmer behaviour. While farmers often highlight the importance of establishing a relationship with their animals [22, 28–29], the traders in the present study emphasised their ability to build relationships with people, i.e. being good at dealing with clients. Hence, being a good trader has less to do with how animals are cared for and more to do with maintaining good customer relations. Influencing client demands is therefore likely to be an efficient way of adjusting traders' perceptions and practices.

The study visits to both markets were conducted at times of comparatively low trade activity. Visiting the markets during the peak trade season would have increased the possibility to observe trader behavior during disease outbreaks, as more animals and people present means increased risk of disease dissemination. At the time of the study visits, both the Lusaka and Kasumbalesa markets were run by the Small Livestock Association of Zambia (SLAZ). Several of the respondent traders, slaughterhouse workers, transporters etc. were members of the association. While this may have reduced respondents' willingness to supply information that could project a negative image of the organisation, we do not believe that this had any significant bearing on their ability to answer the research questions. In this study, the respondent traders were treated as a homogenous group, and differences related to gender, country of origin etc. were not investigated. These are relevant aspects for exploration in future larger studies.

Conclusions

In conclusion, while lack of knowledge is a common explanation in the existing scientific literature as to why traders, through their actions, engage in behaviours that can lead to disease contamination and spread, this study indicates that the reality is far more complex than that. This warrants a shift in the focus of research and outreach projects from simply identifying knowledge gaps to understanding the underlying reasons and drivers behind certain behaviours. Through increased awareness of traders' perceptions and practices, risk mitigation measures and communication strategies relevant for the local context can be formulated. This could reduce the risks of trade and market-associated disease dissemination, which would lead to improved small ruminant health and smallholder farmer livelihoods in Zambia.

Supporting information

S1 File. An overview interview guide for the topics that were covered in the semi-structured interviews with traders.
(DOCX)

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Transboundary diseases in small ruminants are threats to health, livelihoods and society in Zambia and Tanzania. Animal trade and movement, both of which tend to increase close to international borders, are known drivers of disease dissemination. This thesis has combined seroepidemiology and qualitative data strategies to explore the circulation of selected small ruminant transboundary diseases in Zambia and in the Tanzania-Zambia border region, with a focus on the impact of trade and border proximity.

Sara Lysholm received her postgraduate education at the Department of Clinical Sciences, Swedish University of Agricultural Sciences. She obtained her MSc in veterinary medicine in 2017, at the Faculty of Veterinary Medicine and Animal Science at the same university.

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