EPIDEMIOLOGICAL INVESTIGATION OF *PESTE DES PETITS* RUMINANTS IN SELECTED REGIONS OF TANZANIA

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

Peste des petits ruminants (PPR), one of the most economically important disease of small ruminants has been earmarked for eradication following the successful global eradication of rinderpest. The disease is caused by *peste des petits* ruminants virus (PPRV). For eradication to be successful, the different PPR situations and contexts in each region and country must be well understood and reflected upon. In this study, the objective was to conduct an epidemiological assessment of the spread and persistency of PPR in selected areas of Tanzania with focus on distribution of antibodies to PPRV, PPRV genetic diversity and identification of practices by small stock farmers in response to this disease.

The study was carried out using samples collected between 2013 to 2016. Sera samples were collected from clinically healthy sheep and goats for detection of antibodies to PPRV, together with blood, swabs and tissues for detection of the virus using molecular assays. A questionnaire was also administered in order to collect demographic characteristics, knowledge and practices relating to this disease from small ruminant farmers during sample collection. The overall true seroprevalences from samples collected in 2013 and 2015 was 27% (n = 3838) and for samples collected in 2016 was 30% (n = 328). Seroprevalences for samples collected in 2013, 2015 and 2016 show that the disease is continuing to spread in the country as seropositivity was observed in regions where previously no disease had been reported. Presence of the virus was found in samples collected in Morogoro and Arusha regions in 2016. Molecular characterization of the virus clustered them into two lineages, II and III. This confirmed presence of two lineages circulating in animals from the same herd, adding another dimension into the

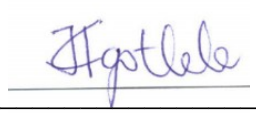
complexity of the disease in Tanzania. Other findings were confirmation of co-infections with *Mycoplasma capricolum* subspecies *capripneumoniae*, *Pasteurella multocida* and *Capripoxvirus* which cause similar clinical signs to PPR, complicating clinical diagnosis but emphasizing the importance of laboratory confirmation. Small ruminant farmers' knowledge by regions on the disease occurrence was found to be high in Arusha region (northern Tanzania) and low in Morogoro region (eastern Tanzania), corresponding with the seroprevalences observed from samples collected in 2013, 2015 and 2016 in the said regions. Risk practices identified during outbreaks included trading of live animals, use of veterinary drugs and unattendance to sick animals. These risk practices could facilitate the spread of the disease in the country especially as the disease is transmitted through contact with infected animals. In conclusion, this study has revealed that PPR continues to spread within Tanzania as evidenced by antibodies to PPRV detected in areas that previously did not have the disease. Presence of two PPRV lineages shows the ability of lineages to co-circulate in an endemic area as well as in the presence of co-infections with other diseases in the local herd.

Overall, there is poor knowledge by small ruminant farmers in the study areas that may be contributing to the spread of PPR. It is therefore recommended that annual vaccinations be carried out after well designed participatory surveillances are conducted to improve the herd immunity to levels that can contain the spread of PPR as a control measure. These vaccinations should take into consideration the geographical distribution of PPR in Tanzania so as to create buffer zones to stop further spread to areas with low or no disease, within and in neighboring countries. Genetic diversity of the virus strains circulating in the country should be further investigated by whole genome sequencing and how they compare to other strains. To prioritize on small ruminant farmer's knowledge

on the disease and emphasize how their participatory disease surveillance can help with the ultimate goal of eradicating PPR in Tanzania, regionally and globally.

DECLARATION

I, TEBOGO KGOTLELE, do hereby declare to the Senate of Sokoine University of Agriculture that, this thesis is my own original work and that it has neither been submitted nor concurrently submitted for a degree award in any other institution.



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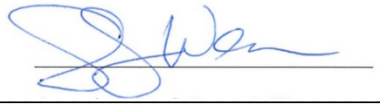

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DEDICATION

To almighty God, my daughter Kagelelo Elinah Kgotlele, my mother Lesedi Kgotlele and all members of my family and friends.

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ORGANISATION OF THE THESIS

This thesis is organized in the "published papers format" and consists of seven chapters as follows;

a. Chapter one is the General Introduction

b. Chapters two to five are the four manuscripts out of the specific objectives;

i. Seroprevalence of *peste des petits* ruminants from samples collected in different regions of Tanzania in 2013 and 2015. Published in *Journal of Veterinary Science and Technology* 7: 394, October 2016 (doi: 10.4172/2157-7579.1000394).

ii. Detection of *peste des petits* ruminants and concurrent secondary diseases in sheep and goats in Ngorongoro district, Tanzania. Published in *Comparative Clinical Pathology* 28(3): 755 – 759, November 2018 (doi: 10.1007/s00580-018-2848-5).

iii. Molecular characterization of *peste des petits* ruminants virus in a farm in Melela Mlandizi village in Tanzania from 2014 to 2015. Manuscript in preparation.

iv. Knowledge and risk practices of small stock farmers on the occurrence of *peste des petits* ruminants in selected areas of Tanzania. Manuscript in preparation

c. Chapter six is the General Discussion

d. Chapter seven is General Conclusion and Recommendations

LIST OF ABBREVIATIONS

ºC	degree Celsius
bp	base pair
BNVL	Botswana National Veterinary Laboratory
BLAST	Basic Local Alignment Search Tool
CaPV	*Capripoxvirus*
CCPP	contagious caprine pleuropneumonia
cELISA	competitive enzyme-linked immunosorbent assay
CI	confidence intervals
CIRAD	Agricultural Research Centre for International Development
Cq	quantification cycle
DNA	deoxyribonucleic acid
DVO	District Veterinary Officer
EMVT	Animal Health and Veterinary Medicine in the Tropics
F	fusion protein
FAO	Food and Agriculture Organization of the United Nations
GREP	Global Rinderpest Eradication Programme
H	haemagglutinin protein
ICTV	International Committee on Taxonomy of Viruses
L	polymerase protein
M	matrix protein
Mccp	*Mycoplasma capricolum* subspecies *capripneumoniae*
MLF	Ministry of Livestock and Fisheries
MLFD	Ministry of Livestock and Fisheries Development

n	sample size
N	nucleoprotein
NCBI	National Center for Biotechnology Information
NP3/NP4	nucleoprotein primer set
OD	optical density
OIE	World Organization for Animal Health
OR	odds ratio
p-value	level of significance
P	phosphoprotein
PCR	polymerase chain reaction
PPR	*peste des petits* ruminants
PPRV	*peste des petits* ruminants virus
PPR GCES	*peste des petits* ruminants Global Control and Eradication Strategy
qRT-PCR	real-time reverse transcription polymerase chain reaction
RNA	ribonucleic acid
SACIDS	Southern African Centre for Infectious Disease Surveillance
SPSS	Statistical Package for Social Sciences
TP	true prevalence
µL	microliter
URT	United Republic of Tanzania

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 *Peste des petits* ruminants in the world

Everyday around the world, consumers enjoy one or more of Africa's vast range of products derived from sheep and goats (OIE and FAO, 2015a). The continent produces approximately 16.6% and 25.4% of the total global sheep and goat meat, respectively (FAO and OIE, 2016). Expert opinion is that these figures are low and that there is potential to increase them. But the potential to increase these figures is hampered by several factors including diseases. Animal diseases are Africa's biggest constraint to achieving production levels that may lead to Africa attaining food and nutritional sufficiency (OIE and FAO, 2015b). One of the major diseases affecting productivity of sheep and goats is *peste des petits* ruminants (PPR), a highly contagious viral disease affecting small ruminants.

The disease was first reported and described in Ivory Coast in 1942 (Gargadennec and Lalanne, 1942). Despite it being considered a West African disease, it has since spread aggressively beyond the borders of West Africa into the rest of Africa and the world (Geerts, 2009; Jilo, 2016). Currently, PPR is present in Central, Eastern and Western Africa, Asia and the Middle East with the risk of spreading further to other regions (Fig. 1.1).

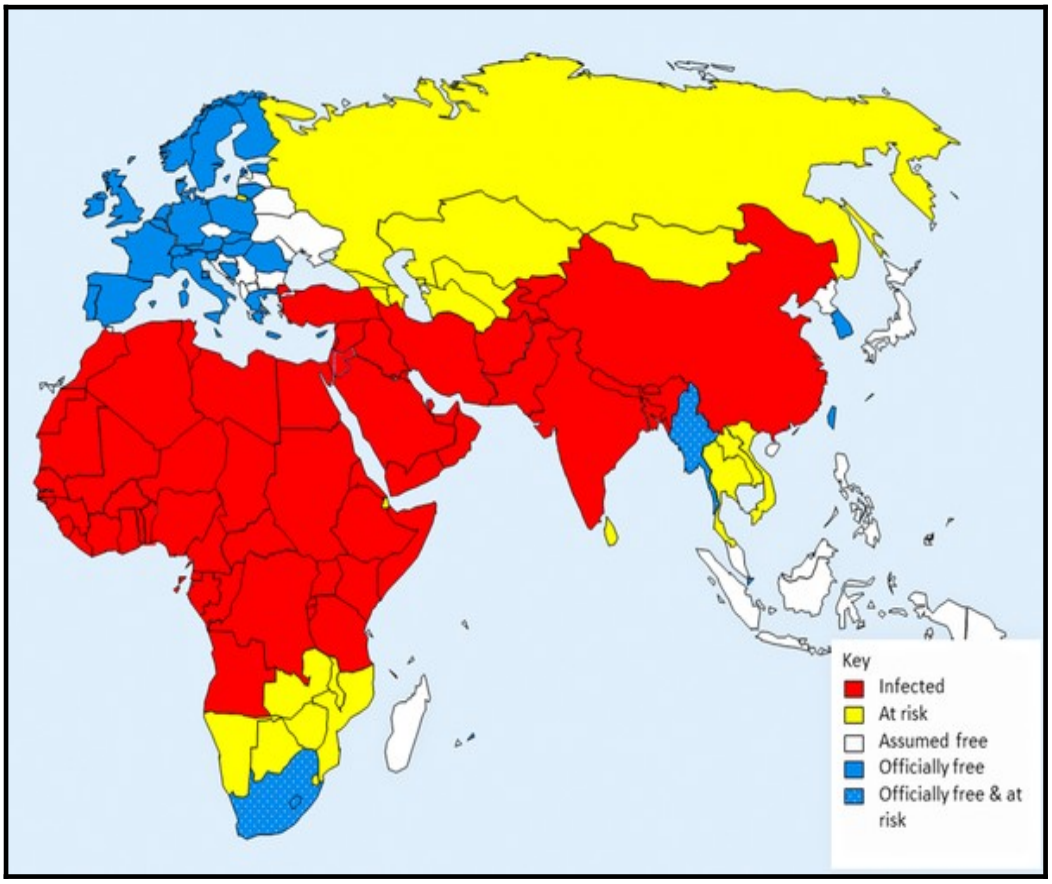

Figure 1.1: Spatial distribution of *peste des petits* ruminants in the world as of 2016 (Source: Jones *et al.,* 2016).

The regions where PPR is endemic are home to approximately 1.7 billion sheep and goats, which is roughly 80% of the global population of sheep and goats (OIE and FAO, 2015b). Sheep and goats are the primary livestock of many low-income, food-deficient prone households and poor livestock keepers in many of the countries that are infected. Thus, the disease is a threat to food and nutritional security, and a danger to people's livelihoods who depend on these animals, especially the world's most vulnerable and marginalized rural communities. Frequently, these are women and children who play an important role in caring for sheep and goats (FAO, 2015).

Globally, the disease has become important because of the successful eradication of rinderpest. *Peste des petits* ruminants and rinderpest share similar characteristics, and their causative agents are members of the same genus (Mornet *et al.*, 1956; Kwiatek *et al.*, 2010). The similarities of the two viruses have led FAO and OIE to mobilize the international community to fight and eradicate PPR by 2030 using the same strategy that led to the successful eradication of rinderpest, the Global Rinderpest Eradication Programme (GREP) (Padhi and Ma, 2014; OIE and FAO, 2015a). One of the main lessons learnt from the GREP was eradicating a disease is not just about controlling epidemics, but combinations of epidemiological studies, husbandry and trade practices are also paramount (FAO and OIE, 2016). The studies would contribute to increased disease awareness, proficient diagnostic tests, awareness-raising campaigns to strengthen passive surveillance and knowledge of legal and illegal movement of livestock and animal products (Jilo, 2016).

1.2 Characterisation of *peste des petits* ruminants

1.2.1 Characteristics of the causative agent

Officially, PPR was first described in 1942 in Ivory Coast by Gargadennec and Lalanne (1942). The original description was made under the name '*peste des espèces ovine et caprine*', meaning plague of ovine and caprine species (Adombi *et al*., 2017). As of September 2016, 76 countries out of 208 countries had reported or suspected presence of PPR (Dhar *et al.,* 2002; Geerts, 2009; Banyard *et al*., 2010; Kwiatek *et al.,* 2011; FAO and OIE, 2016). The aetiological agent of PPR, *peste des petits* ruminants virus (PPRV), was classified in 1979 as a Morbillivirus under the family *Paramyxoviridae* and the order Mononegavirales (Gibbs *et al.,* 1979; Parida *et al.,* 2015). Today, it is classified as

belonging to the species *Small ruminant morbillivirus*, genus *Morbillivirus* in the subfamily *Orthoparamyxovirinae* (Amarasinghe *et al.*, 2019).

The virus genome is linear, single stranded, non-segmented, negative sense RNA of 15,948 nucleotides, the longest of all morbillivirus genomes sequenced so far (Barrett *et al.*, 2005; Diallo *et al.*, 2007). Like other morbilliviruses, the PPRV genome observes "the rule of six" (multiple of six) (Bailey *et al.*, 2007). The six structural viral proteins are 3'-N-P-M-F-H-L-5' (Fig. 1.2) where; N-nucleoprotein, P-phosphoprotein, M-matrix protein, F-fusion protein, H-hemagglutinin, and L-large protein (Sidhu *et al.*, 1993; Luka *et al.*, 2011). The P gene also encodes two non-structural proteins, V and C.

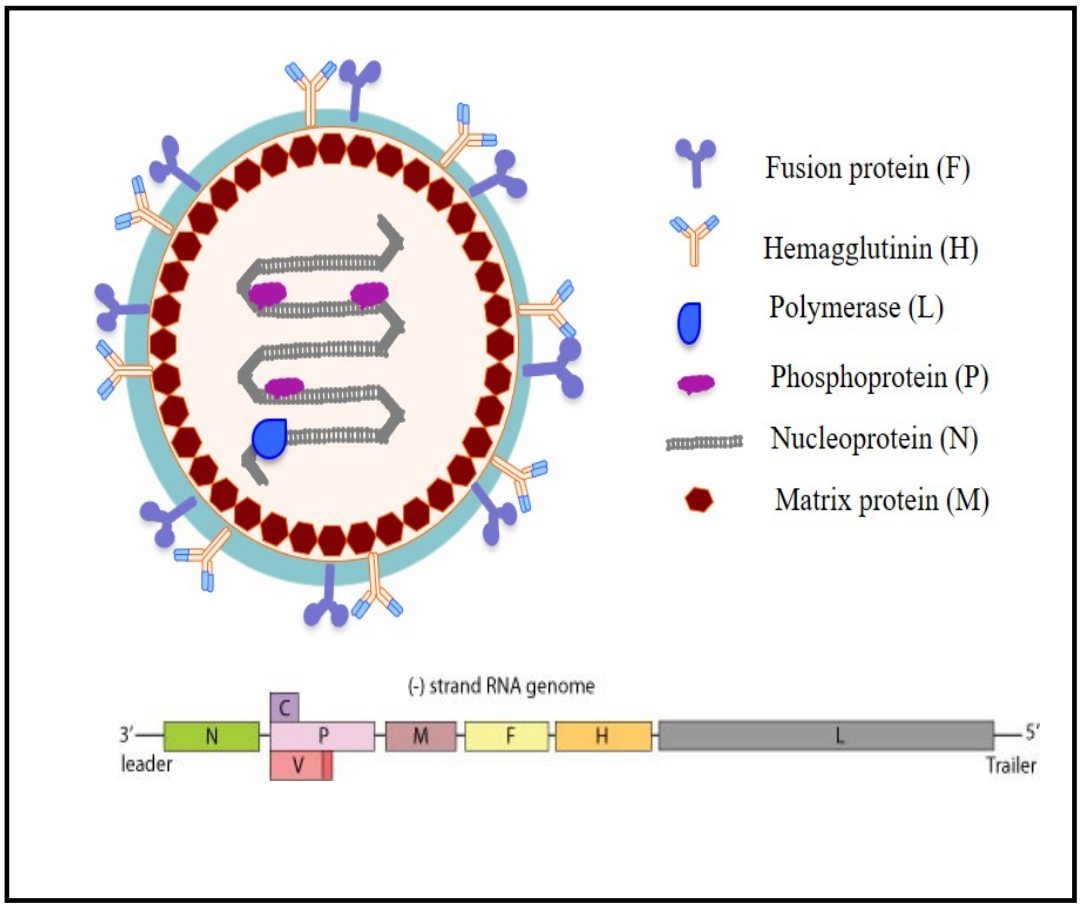

Figure 1.2: *Peste des petits* ruminants virus virion and genome orientation (Author's own).

There is only one serotype of PPRV, but molecular characterization divides it into four genetically distinct lineages (I, II, III and IV) on basis of partial sequence analysis of N and F genes (Esmaelizad *et al.,* 2011; Luka *et al.,* 2011). Lineage I is represented by Western African isolates from the 1970s and recent isolates from Central Africa; lineage II by West African isolates from the Ivory Coast, Guinea and Burkina Faso; lineage III by isolates from Eastern Africa, the Sudan, Yemen and Oman; lineage IV includes viruses isolated from recent outbreaks across the Arabian Peninsula, the Middle East, southern Asia and recently across several African countries (Banyard *et al.*, 2010; Libeau *et al.*, 2014). Some countries have reported presence of more than one lineage in their herds. Examples include Ethiopia, Uganda and United Arab Emirates for lineages III and IV, Nigeria for lineages II and IV, and Tanzania for lineages II, III and IV (Luka *et al.,* 2012; Parida *et al*., 2015; Misinzo *et al*., 2015).

Sheep and goats are the natural hosts of PPRV. Studies from different parts of the world show differences in mortality and morbidity rates of PPRV in both sheep and goats (Balamurugan *et al*., 2015), however, the host range of PPRV extends beyond these species. Camels and several species of wild ungulates have been reported to be infected with PPRV (Furley *et al*., 1987; Ogunsanmi *et al.,* 2003; Abu-Elzein *et al.,* 2004; Kinne *et al*., 2010; Aziz-ul-Rahman *et al*., 2020). Cattle, buffalo and pigs have been reported to develop subclinical infection but do not excrete the virus, thus are not considered to be important in the epidemiology of the virus (Parida *et al*., 2015).

The virus is transmitted by close contact of infected animals with susceptible animals through aerosols or clinical excretions (Roeder *et al.,* 1999; Banyard *et al*., 2010; Abubakar *et al*., 2012). Transmission is also possible through ingestion of contaminated feed and water (Jilo, 2016). There is no carrier state for PPRV, but the virus may be shed during incubation periods and post-recovery state in faeces for 11 weeks after complete recovery (Couacy-Hymann *et al*., 2007; Ezeibe *et al.,* 2008). Therefore, nomadic movements, exchange of animals and change in seasonas are major risk factors contributing to PPRV transmission among goat and sheep populations (Elsawalhy *et al*., 2010; Abubakar *et al*., 2011).

1.2.2 Pathogenesis and clinical signs of *peste des petits* ruminants

Following infection, there is an incubation period that ranges from 3 to 14 days during which the virus replicates in the lymph nodes before spreading to other tissues including the lungs causing primary viral pneumonia (Lefèvre and Diallo, 1990; Bailey *et al*., 2005). Viremia results in dissemination of the virus, leading to severe leucopoenia which facilitates secondary bacterial infection, the main characteristic of pathogenesis of PPRV (Jilo, 2016). Other characteristics include severe purulent ocular discharges, severe purulent nasal discharges, respiratory distress and coughing (Diallo *et al.,* 2007; Kgotlele *et al.,* 2014). Animals may also develop watery, foul smelling, and/or blood-stained diarrhoea accompanied or preceded by sudden drop in core body temperature which is followed by death (CFSPH, 2008). Pregnant animals may abort with morbidity and mortality rates higher in young animals than in adults (Abdalla *et al*., 2012). In cases where infection is mild, animals may recover and return to pre-infection health status within ten to fifteen days of infection (Parida *et al*., 2015). Clinical signs and mortality rates vary depending on virulence of the virus strain and health status of affected animals (OIE, 2013).

1.2.3 Pathology of *peste des petits* ruminants

Post mortem findings as described by Roeder *et al*. (1999) include emaciated carcass with hindquarters soiled with soft/watery faeces. The eyes and nose contain dried-up discharges with eyeballs sunken (Chauhan *et al*., 2009). Erosions are seen on the gums, soft and hard palates, tongue and cheeks (Roeder *et al*., 1999). The pathology of PPR is characterized and dominated by retrogressive and necrotic changes in lymphoid tissues and epithelial cells of gastrointestinal and respiratory systems (Balamurugan *et al.*, 2015). The spleen and lymph nodes, particularly those associated with the respiratory and gastrointestinal tracts are generally congested and enlarged (Kumar *et al.*, 2004). Congested nasal cavity with clear or creamy yellow exudates and lungs with dark red or purple areas firm to the touch (evidence of pneumonia) (Baron *et al.*, 2011). The lymph nodes, particularly those associated with the respiratory and gastrointestinal tracts are generally congested and enlarged (Kumar *et al*., 2004). Characteristic zebra striping or markings may occur in the large intestines, but are not a consistent finding (OIE, 2013).

1.2.4 Diagnosis of *peste des petits* ruminants

The disease can be confused with a number of other diseases of small ruminants because of its similarity in clinical signs due to secondary bacterial infections because of the compromised immunity (OIE, 2013). Diseases that present similar clinical signs include foot-and-mouth disease, bluetongue, pneumonic pasteurellosis, contagious caprine pleuropneumonia, coccidiosis and gastro-intestinal helminth infections (Munir *et al*., 2009; Jilo, 2016). For this reason, laboratory confirmation is necessary to rule out other causes. The laboratory tests currently available for PPR diagnosis are grouped into three categories. Those that detect the antigen (viral proteins), those that detect genetic material and those that detect antibodies (Parida *et al.,* 2015). Laboratory tests that look for the

antigen or genetic material detect acute infections, and those that look for antibodies to the virus are for surveillance studies to estimate how widespread the infection is in a flock or area (Baron *et al*., 2011). The virus may also be isolated from pathological specimens in cell tissue cultures and examined daily for evidence of cytopathic effect (CPE) (Adombi *et al*., 2011).

The efficiency of laboratory diagnosis relies greatly on integrity of samples submitted as the virus is affected by how samples were collected and transported. This is because the virus is very sensitive to temperature and its survival outside the host is very short (Rossiter and Taylor, 1994; Gitao *et al*., 2012).

1.2.5 Treatment and control of *peste des petits* ruminants

There is no specific treatment for PPR but treatment for bacterial and parasitic complications has been noted to decrease mortality in affected herds (Aiello and Moses, 2011). Infection induces strong immune responses resulting in lifelong immunity (Cosby *et al.,* 2006; Jilo, 2016). There are vaccines available for PPR that have been used for decades to control the disease. Currently, there are four live attenuated vaccine strains (Nigeria 75/1, Sungri/96, Arasur/87 and Coimbatore/97) being regularly employed in endemic areas with great success (Sen *et al*., 2010; Parida *et al*., 2015). The Nigeria 75/1 vaccine (lineage II) was developed for Africa but is used worldwide to control PPR in different endemic zones with different lineages. This is because one strain provides cross-protection to another strain of the same or different lineage (Kumar *et al.,* 2014). The other three vaccine strains are of Indian-origin belonging to lineage IV (Saravanan *et al.,* 2010). The attenuated vaccines provide protective immunity in sheep and goats at least for 3 to 4 years, while pregnant animals passing passive immunity to their offspring which protects them for 3 to 5 months (Munir, 2013). The only problem with the vaccines

is that the antibody response induced in animals cannot be distinguished from natural infection by diagnostic tests (Diallo *et al.,* 2007). This is because immunity developed after vaccination lasts from three months to life in vaccinated animals (Kumar *et al.,* 2014; Parida *et al.*, 2015).

1.2.6 Impact of *peste des petits* ruminants on the economy of affected communities

The high mortality rates and prevalence in developing countries means PPR is an economically relevant disease for livestock (OIE and FAO, 2015a). The annual global impacts of PPR have been estimated at between US$1.4 billion and US$2.1 billion due to high mortalities, production loss and control costs (OIE and FAO, 2015b). Countries like India where the disease is endemic, estimated losses were US$180 million in 2014/2015 (OIE and FAO, 2015b). Data available from Kenya, Tanzania and Ivory Coast show that mortality due to PPR directly depleted affected households of small ruminant's asset base by 28% to 68% (FAO and OIE, 2016). Other associated losses included culling or distress sales at reduced prices of up to half the normal market price of sheep and goats, and raising poverty levels by 10% (Jones *et al*., 2016). Thus, these figures highlight the economic importance of PPR in areas where livelihoods highly depend on livestock, particularly sheep and goats.

1.2.7 Global strategy for the control and eradication plan for *peste des petits* ruminants

The PPR Global Control and Eradication Strategy (PPR GCES) was endorsed at the international conference for the control and eradication of PPR organized by FAO and OIE held in Abidjan, Ivory Coast in 2015 (OIE and FAO, 2015b). The PPR GCES proposed a

plan that would lead to PPR eradication by 2030 (Parida *et al.*, 2015). The main objective of the strategy is to progressively reduce the incidence and spread of PPR and ultimately eradicate PPR from infected countries and ensure that previously non-infected countries remain free from the disease (OIE and FAO, 2015a). In the strategy, good quality veterinary services have been identified as one of the integrated components that is indispensable for the successful and sustainable implementation of PPR prevention and control activities worldwide (FAO and OIE, 2016). Strengthening of veterinary services is a cornerstone of the strategy which will provide the necessary enabling environment to control other animal diseases as well. Eradication would lead to societal impacts and outcomes that include improved contribution of the small ruminant sector to food security and nutrition, public health and significant reduction in poverty through enhanced livelihoods of over 330 million poor livestock farmers in Africa, the Middle East and Asia (OIE and FAO, 2015a).

1.3 Tanzania and *peste des petits* ruminants

1.3.1 Livestock sector and contribution of small ruminants in Tanzania

Tanzania is located on the eastern side of the African continent bordered by Kenya and Uganda to the north; Burundi, Rwanda and Democratic Republic of the Congo to the west; Zambia, Malawi and Mozambique to the south with the Indian Ocean on the east. The country has the third largest livestock population on the African continent comprising 16.7 million goats and 8 million sheep, complimented by 25 million cattle and 2.4 million pigs (URT, 2015). In Tanzania, the livestock industry is categorized into two major productions, the intensive and extensive systems (Mlote *et al.*, 2013). The intensive system is characterised by high livestock population with intense agricultural activity which is market oriented while the extensive system is mostly agro-pastoralism and pastoralism (Kusiluka and Kambarage, 1996). About 80% of the livestock farmers in

Tanzania use the extensive system which is mostly constrained by poor animal husbandry practices, lack of modernization, and lack of market orientation (URT, 2013). Despite these constraints, the extensive system has sustained the livelihood of the pastoral communities for many decades (URT, 2006). It is reported that 60% of rural households in Tanzania engage in livestock keeping, earning an average of over 20% of their income from livestock and benefiting from other livestock uses (Covarrubias *et al.,* 2012). Overall, the livestock industry's contribution to the country's GDP is low as the sector is severely constrained by several factors including livestock diseases (URT, 2006; URT, 2015).

1.3.2 *Peste des petits* ruminants in Tanzania

The country was officially declared to be infected with PPR in 2008 after high mortality rates and clinical signs suspect of PPR were observed in sheep and goats in Ngorongoro district (Kivaria *et al.,* 2009; Karimuribo *et al*., 2011; Kivaria *et al*., 2013). The disease was suspected to have spread from Uganda and Kenya due to the uncontrolled transnational livestock movement across borders by pastoralists (Swai *et al.,* 2009; Gitao *et al.,* 2012). Following the official confirmation, PPR vaccination campaigns were carried out around the 2008 outbreak foci (Arusha region), eastern and southern Tanzania (Morogoro and Mtwara regions respectively) in 2008 and 2010 (URT, 2013). Vaccinating sheep and goats is known to be the most effective control measure as the vaccines provide lifelong immunity (Muniraju *et al*., 2014). It has also been proven that control in endemic areas relies mainly on vaccinations (Waret-Szkuta *et al*., 2008).

Despite the vaccinations carried out, by the end of 2012 reports of the disease were coming from the eastern and southern parts of the country, indicating the downward spread of the disease from north to south (Muse *et al*., 2012). Currently, PPR is endemic

in Tanzania with studies showing presence of the disease in the north, east, central and south of the country (Swai *et al*., 2009; Karimuribo *et al*., 2011; Kivaria *et al*., 2013; Torsson *et al*., 2016). Based on the nucleoprotein gene, there are three lineages of PPRV that have been found to be circulating in Tanzania. These are lineage III found in outbreaks in northern and eastern regions, and lineages II and IV in the southern regions (Kivaria *et al.,* 2013; Kgotlele *et al.,* 2014; Misinzo *et al.,* 2015).

1.4 Study justification and objectives

1.4.1 Justification of the study

Peste des petits ruminants continues to spread in Tanzania despite the availability of an effective vaccine and knowledge of its presence for the past 10 years. The spread of PPR is a problem to Tanzania as the country joins the international community towards achieving the PPR GCES objectives of reducing incidence and spread of the disease and ultimately eradication by 2030 in the national herd. Sheep and goat farming in Tanzania is an important economic activity being practiced by about 30% of the agricultural households and contributing about 22% to the national meat supplies (URT, 2006). The animals are widely distributed and adapted to many agro-ecological zones found in the country and their ability to multiply and grow faster than cattle at relatively low cost makes them more attractive to small-scale farmers (Kivaria *et al.,* 2013). The reduction of incidence of PPR and its ultimate eradication would be one less threat to sheep and goats as well as eliminating a threat to the livelihoods of many households especially the rural poor. Thus, the study is aimed at creating new knowledge on PPR in Tanzania with a focus on the epidemiological features of the causative agent of the disease and identifying gaps that would counteract the control measures put in place to meet the goals of eventually eradicating the disease in the national herd.

1.4.2 Main objective

The overall objective of this study was to investigate the occurrence of PPR among sheep and goats in selected areas of Tanzania and assess how small stock farmers understand and respond to the threat posed by PPR, an emerging disease of socio-economic importance and trade importance.

1.4.3 Specific objectives

i. To determine the seroprevalence of PPR from previously collected samples in selected areas of Tanzania,
ii. To examine the presence of PPRV infection in sheep and goats in selected areas of Tanzania,
iii. To identify circulating lineages of PPRV in selected areas of Tanzania, and
iv. To determine awareness and practices of small stock farmers regarding PPR in selected areas of Tanzania.

1.5 Ethics statement

The study was approved by the Scientific Committee of Sokoine University of Agriculture (SUA) and permission to carry out the study in the different regions sought from the District Executive Directors. Farmers' verbal consents were also sought before the start of study.

1.6 References

Abdalla, A. S., Majok, A. A., El Malik, K. H. and Ali, A. S. (2012). Sero-prevalence of *peste des petits* ruminants virus (PPRV) in small ruminants in Blue Nile, Gadaref and North Kordofan States of Sudan. *Journal of Public Health and Epidemiology* 4(3): 59 - 64.

Abubakar, M., Rajput, Z. I., Arshed, M. J., Sarwar, G. and Ali, Q. (2011). Evidence of *peste des petits* ruminants virus (PPRV) infection in Sindh Ibex (*Capra aegagrus blythi*) in Pakistan as confirmed by detection of antigen and antibody. *Tropical Animal Health and Production* 43(4): 745 - 747.

Abubakar, M., Mehmood, F., Jeelani, A. and Arshed, M. J. (2012). Application of PCR in diagnosis of *peste des petits* ruminants virus (PPRV), polymerase chain reaction. [http://www.intechopen.com/ books /polymerase -chain-eaction/ application -of-pcr-in-diagnosis-of-peste-des-petits-ruminants-virus-pprv-] site visited on 5/7/2012.

Abu-Elzein, E. M., Housawi, F. M., Bashareek, Y., Gameel, A. A., Al-Afaleq, A. I. and Anderson, E. (2004). Severe PPR infection in gazelles kept under semi-free-range conditions. *Journal of Veterinary Medicine, Infectious Diseases and Veterinary Public Health* 51(2): 68 - 71.

Adombi, C. M., Lelenta, M., Lamien, C. E., Shamaki, D., Yao, M. K., Traore, A., Silber, R., Couacy-Hymann, E., Bodjo, S. C., Djaman, J. A., Luckins, A. G. and Diallo, A. (2011). Monkey CV1 cell line expressing the sheep-goat SLAM protein: a highly sensitive cell line for the isolation of *peste des petits* ruminants virus from pathological specimens. *Journal of Virological Methods* 173(2): 306 - 313.

Adombi, C. M., Waqas, A. G., Dundon, W., Li, S., Daojin, Y., Kakpo, L., Aplogan, G. L., Diop, M., Lo, M. M., Silber, R., Loitsch, A. and Diallo, A. (2017). *Peste des petits* ruminants in Benin: Persistence of a single virus genotype in the country for over 42 Years. *Transboundary and Emerging Diseases* 64(4): 1037 - 1044.

Aiello, S. E. and Moses, M. A. (2011). The Merck Veterinary Manual online. [http://www.merckmanuals.com/vet/search.html?qt=peste+des+petits+ruminants&start=1&context=%2Fvet] site visited on 5/3/2012.

Amarasinghe, G. K., Ayllòn, M. A., Báo, Y., Basler, C. F., Bavari, S., Blasdell, K. R., Briese, T., Brown, P. A., Bukreyev, A., Balkema-Buschmann, A., Buchholz, U. J., Chabi-Jesus, C., Chandran, K., Chiapponi, C., Crozier, I., de Swart, R. L., Dietzgen, R. G., Dolnik, O., Drexler, J. F., Dürrwald, R., Dundon, W. G., Duprex, W. P., Dye, J. M., Easton, A. J., Fooks, A. R., Formenty, P. B. H., Fouchier, R. A.M., Freitas-Astúa, J., Griffiths, A., Hewson, R., Horie, M., Hyndman, T. H., Jiāng, D., Kitajima, E. W., Kobinger, G. P., Kondō, H., Kurath, G., Kuzmin, I. V., Lamb, R. A., Lavazza, A., Lee, B., Lelli, D., Leroy, E. M., Lǐ, J., Maes, P., Marzano, S. L., Moreno, A., Mühlberger, E., Netesov, S. V., Nowotny, N., Nylund, A., Økland, A. L., Palacios, G., Pályi, B., Pawęska, J. T., Payne, S. L., Prosperi, A., Ramos-González, P. L., Rima, B. K., Rota, P., Rubbenstroth, D., Shī, M., Simmonds, P., Smither, S. J., Sozzi, E., Spann, K., Stenglein, M. D., Stone, D. M., Takada, A., Tesh, R. B., Tomonaga, K., Tordo, N., Towner, J. S., van den Hoogen, B., Vasilakis, N., Wahl, V., Walker, P. J., Wang, L. F., Whitfield, A. E., Williams, J. V., Zerbini, F. M., Zhāng, T., Zhang, Y. Z. and Kuhn, J. H. (2019). Taxonomy of the order Mononegavirales: update 2019. *Archives of Virology* 164(7): 1967 - 1980.

Aziz-Ul- Rahman, Dhama, K., Ali, Q., Hussain, I., Oneeb, M., Chaudhary, U., Wensman, J. J. and Shabbir, M. Z. (2020). *Peste des petits* ruminants in large ruminants, camels and unusual hosts. *Veterinary Quarterly* 40(1): 35-42.

Bailey, D., Banyard, A., Dash, P., Ozkul, A. and Barrett, T. (2005). Full genome sequence of *peste des petits* ruminants virus, a member of the *Morbillivirus* genus. *Virus Research* 110(1-2): 119 - 124.

Balamurugan, V., Rahman, H. and Munir, M. (2015). Host susceptibility to *peste des petits* ruminants virus, In: Munir, M. (Ed), *Peste des petits* ruminants virus, Springer-Verlag Berlin Heidelberg. pp. 39 - 50.

Bailey, D., Chard, L. S., Dash, P., Barrett, T. and Banyard, A. C. (2007). Reverse genetics for *peste des petits* ruminants virus (PPRV): Promoter and protein specificities. *Virus Research* 126(2): 250 - 255.

Banyard, A. C., Parida, S., Batten, C., Oura, C., Kwiatek, O. and Libeau, G. (2010). Global distribution of *peste des petits* ruminants and prospects for improved diagnosis and control. *Journal of General Virology* 91(12): 2885 - 2897.

Baron, M. D., Parida, S. and Oura, C. A. L. (2011). *Peste des petits* ruminants: A suitable candidate for eradication? *The Veterinary Record* 169(1): 16 - 21.

Barrett, T., Pastoret, P. P. and Taylor, W. P. (2005). *Rinderpest and Peste Des Petits Ruminants: Virus Plagues of Large and Small Ruminants.* Academic Press, London. 341pp.

CFSPH (2008). *Peste des petits* ruminants. [http://www.cfsph .iastate.edu/Factsh eets/pdfs/peste_des_petits_ruminants.pdf] site visited on 4/3/2012.

Chauhan, H. C., Chandel, B. S., Kher, H. N., Dadawala, A. I. and Agrawal, S. M. (2009). *Peste des petits* ruminants virus infection in animals. *Veterinary World* 2(4): 150 - 155.

Cosby, S. L., Chieko, K. and Yamaguchi, K. (2006). Rinderpest and *peste des petits* ruminants virus: Plague of large and small ruminants. In: *Biology of Animal Infections.* Academic Press, London. pp. 197 - 221.

Couacy-Hymann, E., Bodjo, C., Danho, T., Libeau, G. and Diallo, A. (2007). Evaluation of the virulence of some strains of *peste des petits* ruminants virus (PPRV) in experimentally infected West African dwarf goats. *Veterinary Journal* 173(1): 178 - 183.

Covarrubias, K., Nsiima, L. and Zezza, A. (2012). *Livestock Farming and Livelihoods in Rural Tanzania. A Descriptive Analysis of the 2009 National Panel Survey*, Food and Agriculture Organization, Rome, Italy.

Dhar, P., Sreenivasa, B. P., Barrett, T., Corteyn, M., Singh, R. P. and Bandyopadhyay, S. K. (2002). Recent epidemiology of *peste des petits* ruminants virus. *Veterinary Microbiology* 88(2): 153 - 159.

Diallo, A., Minet, C., Goff, C., Berhe, G., Albina, E., Libeau, G., Barrett, T. and le Goff, C. (2007). The threat of *peste des petits* ruminants: progress in vaccine development for disease control. *Vaccine* 25(30): 5591 - 5597.

Elsawalhy, A., Mariner, J. C., Chibeu, D., Wamwayi, H., Wakhusama, S., Olaho-Mukani, W. and Toye, P. (2010). Pan African strategy for the progressive control of *peste des petits* ruminants (Pan African PPR strategy). *Bulletin of Animal Health and Production* in *Africa* 58(3): 185-193.

Esmaelizad, M., Jelokhani-Niaraki, S. and Kargar-Moakhar, R. (2011). Phylogenetic analysis of *peste des petits* ruminants virus (PPRV) isolated in Iran based on partial sequence data from the fusion (F) protein gene. *Turkey Journal of Biology* 35: 243 - 247.

Ezeibe, M. C., Okoroafor, O. N., Ngene, A. A., Eze, J. I., Eze, I. C. and Ugonabo, J. A. (2008). Persistent detection of *peste de petits* ruminants antigen in the faeces of recovered goats. *Tropical Animal Health and Production* 40(7): 517 - 519.

FAO (2015). Poster [www.fao.org/ppr, BC274e/1/12.15] site visited on 18/4/2018.

FAO and OIE (2016). *Peste des Petits Ruminants Global Eradication Programme; Contributing to Food Security, Poverty Alleviation And Resilience - Five Years (2017–2021*). Food and Agriculture Organization, Rome. 62pp.

Furley, C. W., Taylor, W. P. and Obi, T. U. (1987). An outbreak of *peste des petits* ruminants in a zoological collection. *The Veterinary Record* 121(19): 443 - 447.

Gargadennec, L. and Lalanne, A. (1942). *La peste des petits* ruminants (The plague of small ruminants). *Bulletin des* Services Zootechniques et des Epizooties del'Afrique Occidentale Francaise. *Bulletin of Zootechnical Services and Epizootics 5*: 16 - 21.

Geerts, S. (2009). Goat *Plague Or Peste Des Petits Ruminants. EAZWV Transmissible Disease Fact Sheet* 25. Institute of Tropical Medicine, Antwerp, Belgium. 3pp.

Gibbs, E. P., Taylor, W. P., Lawman, M. J. and Bryant, J. (1979). Classification of *peste des petits* ruminants virus as the fourth member of the genus morbillivirus. *Intervirology* 11: 268 - 274.

Gitao, G. C., Kihu, S. M., Bebora, L. C., Njenga, J. M., Wairire, G. G., Maingi, N., Muse, E. A., Karimuribo, E. D., Misinzo, G., Mellau, L. S. B., Msoffe, P. L. M., Swai, E. S. and Albano, M. O. (2012). Comparison of *peste des petits* ruminants disease between Tanzania and Kenya, In: *Third RUFORUM Biennial* Meeting. 24 - 28 September 2012, Entebbe, Uganda. pp. 523 - 528.

Jilo, K. (2016). *Peste des petits* ruminants (PPR): Global and Ethiopian aspects, a standard review. *Global Veterinaria* 17(2): 142 - 153.

Jones, B. A., Rich, K. M., Mariner, J. C., Anderson, J., Jeggo, M., Thevasagayam, S., Cai, Y., Peters, A. R. and Roeder, P. (2016). The economic impact of eradicating *peste des petits* ruminants: A benefit-cost analysis. *PLoS One* 11(2): e0149982.

Karimuribo, E. D., Loomu, P. M., Mellau, L. S. B. and Swai, E. S. (2011). Retrospective study on sero-epidemiology of *peste des petits* ruminants before its official confirmation in northern Tanzania in 2008. *Research Opinions in Animal and Veterinary Sciences* 1(3): 184 - 187.

Kgotlele, T., Macha, E. S., Kasanga, C. J., Kusiluka, L. J., Karimuribo, E. D., van Doorsselaere, J., Wensman, J. J., Munir, M. and Misinzo, G. (2014). Partial genetic characterization of *peste des petits* ruminants virus from goats in northern and eastern Tanzania. *Transboundary and Emerging Diseases* 61(1): 56 - 62.

Kinne, J., Kreutzer, R., Kreutzer, M., Wernery, U. and Wohlsein, P. (2010). *Peste des petits* ruminants in Arabian wildlife. *Epidemiology and infection* 138(8): 1211 - 1214.

Kivaria, F. M., Kwiatek, O., Kapaga, A. M., Geneviève, L., Mpelumbe-Ngeleja, C. A. R. and Tinuga, D. K. (2009). Serological and virological investigations on an emerging *peste des petits* ruminants virus infection in goats and sheep in Tanzania, In: *Proceedings of The 27th Tanzania Veterinary Association Scientific Conference*. 4 - 6 December 2009, Arusha, Tanzania.

Kivaria, F. M., Kwiatek, O., Kapaga, A. M., Swai, E. S., Libeau, G., Moshy, W., Mbyuzi, A. O. and Gladson, J. (2013). The incursion, persistence and spread of *peste des petits* ruminants in Tanzania: Epidemiological patterns and predictions. *Onderstepoort Journal of Veterinary Research* 80(1): 593.

Kumar, P., Tripathi, B. N., Sharma, A. K., Kumar, R., Sreenivasa, B. P., Singh, R. P., Dhar, P. and Bandyopadhyay, S. K. (2004). Pathological and immunohistochemical study of experimental *peste des petits* ruminants virus

infection in goats. *Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health* 51(4): 153 - 159.

Kumar, N., Maherchandani, S., Kashyap, S. K., Singh, S. V., Sharma, S., Chaubey, K. K. and Ly, H. (2014). *Peste des petits* ruminant's virus infection of small ruminants: A comprehensive review. *Viruses* 6(6): 2287 - 2327.

Kusiluka, L. and Kambarage, D. (1996). Diseases of small ruminants in sub-Saharan Africa: A handbook common diseases of sheep and goats in sub-Saharan Africa. VETAID, ISBN 09522299 5, 116pp.

Kwiatek, O., Keita, D., Gil, P., Fernandez-Pinero, J., Clavero, M. A. J., Albina, E. and Libeau, G. (2010). Quantitative one-step real-time RT-PCR for the fast detection of the four genotypes of PPRV. *Journal of Virological Methods* 165(2): 168 - 177.

Kwiatek, O., Yahia, H. A., Intisar, K. S., Khalafalla, A. I., Osama, I. M., Obeida, A. A., Magdi, B. A., Osman, H. M., Taha, K. M., Zakia, A., El Harrak, M., Lhor, Y., Diallo, A., Lancelot, R., Albina, E. and Libeau, G. (2011). Asian lineage of *peste des petits* ruminants virus in Africa. *Emerging Infectious Diseases* 17(7): 1223 - 1231.

Lefèvre, P. C. and Diallo, A. (1990). *Peste des petits* ruminants. *Revue Scientifique et Technique de l`Office International des Epizooties. Scientific and Technical Review of the Office International des Epizooties*) 9(4): 935 - 981.

Libeau, G., Diallo, A. and Parida, S. (2014). Evolutionary genetics underlying the spread of *peste des petits* ruminants virus. *Animal Frontiers* 4(1): 14 - 20.

Luka, P. D., Erume, J., Mwiine, F. N., Ayebazibwe, C. and Shamaki, D. (2011). Molecular characterization and phylogenetic study of *peste des petits* ruminants

viruses from north central states of Nigeria. *BioMed Central Veterinary Research* 7(1): 32.

Luka, P. D., Erume, J., Mwiine, F. N. and Ayebazibwe, C. (2012). Molecular characterization of *peste des petits* ruminants virus from Karamoja region of Uganda (2007 - 2008). *Archives of Virology* 157(1): 29 - 35.

Misinzo, G., Kgotlele, T., Muse, E. A., van Doorsselaere, J., Berg, M. and Munir, M. (2015). *Peste des petits* ruminants virus lineage II and IV from goats in southern Tanzania during and outbreak in 2011. *British Journal of Virology* 2(1): 1 - 4.

Mlote, S. N., Mdoe, N. S. Y., Isinika, A. C. and Mtenga, L. A. (2013). Profitability analysis of small scale beef cattle fattening in the Lake Zone in Tanzania. *Journal of Agricultural Economics and Development* 2(5): 203-216.

Mornet, P., Orue, J., Gilbert, Y., Thiery, G. and Sow, M. (1956). *La peste des petits ruminants en Afrique Occidentale Française, ses rapports avec la peste bovine* (*Peste des petits* ruminants in French West Africa, its relationship to rinderpest). *Revue D'Elevage Et De Medecine Veterinaire Des Pays Tropicaux. Review of Livestock and Veterinary Medicine in Tropical Countries* 9(4): 313 - 342.

Munir, M. (2013). *Mononegaviruses of Veterinary Importance, Pathobiology and Molecular Diagnosis*. Coomonwealth for Agricrlture Bureau International: Oxfordshire, UK. pp. 65 - 98.

Munir, M., Abubakar, M., Khan, M. T. and Abro, S. H. (2009). Comparative efficacy of single radial haemolysis test and countercurrent immune electroosmophoresis with monoclonal antibodies-based competitive ELISA for the serology of *peste des petits* ruminants in sheep and goats. *Bulgarian Journal of Veterinary Medicine* 12(4): 246 - 253.

Muniraju, M., Munir, M., Parthiban, A. R., Banyard, A. C., Bao, J. Y., Wang, Z. L., Ayebazibwe, C., Ayelet, G., El Harrak, M., Mahapatra, M., Libeau, G., Batten, C. and Parida, S. (2014). Molecular evolution of *peste des petits* ruminants virus. *Emerging Infectious Diseases* 20(12): 2023 - 2033.

Muse, E. A., Karimuribo, E. D., Gitao, G. C., Misinzo, G., Mellau, L. S., Msoffe P. L. M., Swai, E. S. and Albano, M. O. (2012). Epidemiological investigation into the introduction and factors for spread of *peste des petits* ruminants, southern Tanzania. *Onderstepoort Journal of Veterinary Research* 79(2): 457.

Ogunsanmi, A. O., Awe, E. O., Obi, T. U. and Taiwo, V. O. (2003). *Peste des petits* ruminants (PPR) virus antibodies in African grey duiker (*Sylvicapra grimmia*). *African Journal of Biomedical Research* 6(1): 59 - 61.

OIE (2013). Manual of diagnostic tests and vaccines for terrestrial animals. [http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.11_PPR.pdf] site visited on 22/5/2012.

OIE and FAO (2015a). Global control and eradication of *peste des petits* ruminants: Investing in veterinary systems, food security and poverty alleviation. 28pp.

OIE and FAO (2015b). *Global Strategy for the Control and Eradication of and OIE International Conference For The Control And Eradication Of Peste Des Petits Ruminants.* Food and Agriculture Organization, Abidjan. 88pp.

Padhi, A. and Ma, L. (2014). Genetic and epidemiological insights into the emergence of *peste des petits* ruminants virus across Asia and Africa. *Scientific Reports* 4: 7040pp.

Parida, S., Muniraju, M., Mahapatra, M., Muthuchelvan, D., Buczkowski, H. and Banyard, A. C. (2015). *Peste des petits* ruminants. *Veterinary Microbiology* 181(2): 90 - 106.

Roeder, P. L., Obi, T. U., Taylor, W. and Diallo, A. (1999). *Recognizing Peste Des Petits Ruminants: A Field Manual. EMPRES (Livestock) Animal Health Service.* Food and Agriculture Organization, Rome.

Rossiter, P. B. and Taylor, W. P. (1994). *Peste Des Petits Ruminants. In Infectious Diseases Of Livestock.* Oxford University Press, Cape Town. pp. 26-45.

Saravanan, P., Sen, A., Balamurugan, V., Rajak, K. K., Bhanuprakash, V., Palaniswami, K. S., Nachimuthu, K., Thangavelu, A., Dhinakarraj, G., Hegde, R. and Singh, R. K. (2010). Comparative efficacy of *peste des petits* ruminants vaccines. *Biologicals* 38(4): 479 - 485.

Sen, A., Saravanan, P., Balamurugan, V., Rajak, K. K., Sudhakar, S. B., Bhanuprakash, V., Parida, S. and Sinngh, R. K. (2010). Vaccines against *peste des petits* ruminants virus. *Expert Review of Vaccines* 9(7): 785 - 796.

Sidhu, M. S., Husar, W., Cook, S. D., Dowling, P. C. and Udem, S. A. (1993). Canine distemper terminal and intergenic non-protein coding nucleotide sequences: completion of the entire CDV genome sequence. *Virology* 193(1): 66 - 72.

Swai, E. S., Kapaga, A., Kivaria, F., Tinuga, D., Joshua, G. and Sanka, P. (2009). Prevalence and distribution of *peste des petits* ruminants virus antibodies in various districts of Tanzania. *Veterinary Research Communications* 33(8): 927 - 936.

Torsson, E., Kgotlele, T., Berg, M., Mtui-Malamsha, N., Swai, E. S., Wensman, J. J. and Misinzo, G. (2016). History and current status of *peste des petits* ruminants virus in Tanzania. *Infection Ecology and Epidemiology* 6: 32701.

URT (2006). *National Livestock Policy*. Ministry of Livestock Development, Dar es Salaam. 4pp.

URT (2013). *Peste des petits Ruminants Progressive Control and Eradication Strategy*. Ministry of Livestock and Fisheries Development, Dar es Salaam. 4pp.

URT (2015). *Tanzania Livestock Modernization Initiatives.* Ministry of Livestock and Fisheries Development, Dar es Salaam. 2pp

Waret-Szkuta, A., Roger, F., Chavernac, D., Yigezu, L., Libeau, G., Pfeiffer, D. U. and Guitian J. (2008). *Peste des petits* ruminants (PPR) in Ethiopia: Analysis of a national serological survey. *BioMed Central Veterinary Research* 4: 34.

CHAPTER TWO

2.0 SEROPREVALENCE OF *PESTE DES PETITS* RUMINANTS VIRUS FROM SAMPLES COLLECTED IN DIFFERENT REGIONS OF TANZANIA IN 2013 AND 2015

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2.1 Abstract

Sero-surveillance was conducted to determine seroprevalence of *peste des petits* ruminants virus (PPRV) in sheep and goats population of Tanzania using samples collected in 2013 and 2015. A total of 3838 samples were collected from villages in 14 of the 25 mainland regions. Samples were tested by competitive ELISA for detection of antibodies to PPRV. Overall, 998 of the samples were found to be positive for antibodies to PPRV, giving an overall true seroprevalence of 27.1%. In this study, there was no statistical difference of exposure to PPR between sheep and goats (odds ratio of 1.06, $CI_{95\%}$: 0.89-1.25). The seroprevalence indicates that PPRV is prevalent in small ruminants in the study areas. The study also confirms the presence of antibodies to PPR in sheep and goats in regions of Tanzania that previously had little to no data on the disease, an indication that PPR is spreading within Tanzania with the possibility of spreading to neighboring countries.

Keywords: *Peste des petits* ruminants; Seroprevalence; cELISA; Tanzania

2.2 Introduction

Peste des petits ruminants (PPR) is a highly contagious viral disease of goats and sheep characterized by oculo-nasal discharges, stomatitis, diarrhea and pneumonia (Rahman *et al*., 2016). It is a disease of economic significance because of its transboundary nature, high morbidity and mortality rates which result in loss of production, limitations on export and threat to human food chain (Zahur *et al*., 2011). The disease is caused by *peste des petits* ruminants virus (PPRV), belonging to the genus Morbillivirus of the family *Paramyxoviridae*. The virus is highly contagious and easily transmitted by direct contact through secretions and/or excretions of infected animals (Banyard *et al*., 2010). *Peste des petits* ruminants was first reported in West Africa in the early 1940s and later recognized as endemic in both West and Central Africa (Gargadennec and Lalanne, 1942; Abraham *et al.,* 2005). Currently, PPR is present in Central, Eastern and Western Africa, Asia, and the Near and Middle East (Geerts, 2009). In East Africa, PPR was detected serologically in Kenya and Uganda in 2007 (Libeau *et al*., 2014). Efforts to determine presence of PPR in sheep and goats in Tanzania can be traced back to Loliondo in 1995 through grey literature (Karimuribo *et al*., 2011). Three years later in 1998, the presence of antibodies against PPR was ruled out by a comprehensive study that did not find any antibodies against PPR in Tanzania sheep and goats (Wambura, 2000). A retrospective study done in Ngorongoro district using samples collected for Rift Valley fever virus and PPR surveys showed presence of antibodies against PPR in samples collected in 2004, suggesting the presence of PPRV at that period (Karimuribo *et al*., 2011).

PPR was officially confirmed in Tanzania in 2008 and it was confined to the northern zone in districts bordering Kenya (Kivaria *et al*., 2009; Swai *et al*., 2009). This follows the official confirmation of PPR in neighboring Kenya in 2007 (Libeau *et al*., 2014). The possible spread from Kenya to Tanzania may have been due to the difficulty in

controlling transnational livestock movements across borders, especially where Maasai pastoralists are found on either side (Kivaria *et al.,* 2013). In 2011, an outbreak of PPR was reported in southern Tanzania (Muse *et al*., 2012). In other areas of Tanzania, limited to no data is available about the disease. Therefore, the objective of this study was to determine seroprevalence of PPR in selected regions of Tanzania to have a current comprehensive view about the distribution of PPR in the country.

2.3 Materials and methods

2.3.1 Samples

A total of 3838 serum samples from 118 villages collected from sheep and goats in 14 regions of Tanzania (Fig. 2.1) in 2013 and 2015 were used (Table 2.1). Samples were collected from apparently healthy animals that did not show any clinical signs associated with PPR. These serum samples were submitted to Sokoine University of Agriculture for official confirmation of PPR in Tanzania before a PPR vaccination campaign. Unfortunately, sex of sampled animals could not be retrieved from information stated in the submission forms. Retrieved information was geographical regions, villages and species.

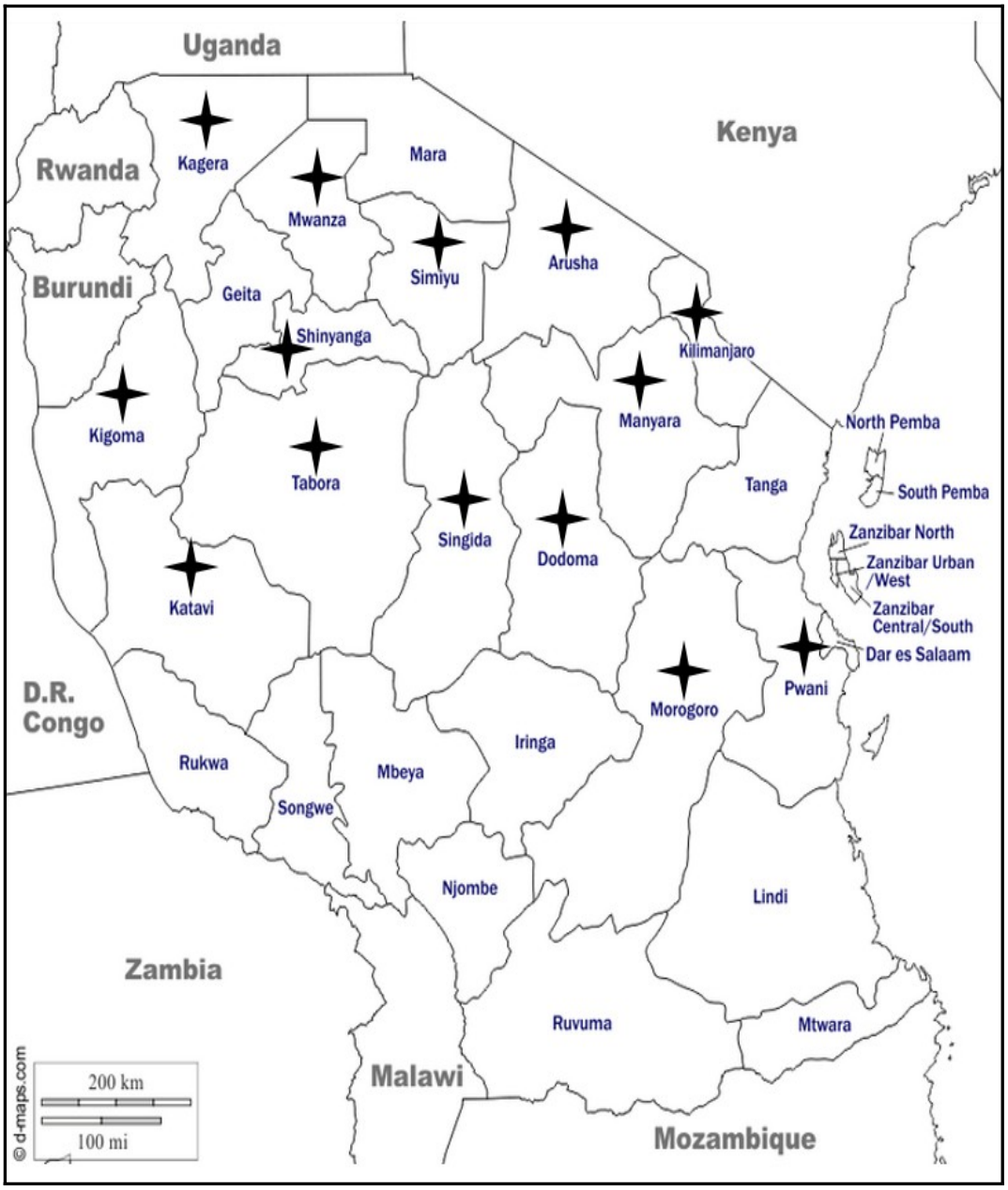

Figure 2.1: Map of Tanzania showing study regions (with stars) in 2013 and 2015.

Table 2.1: Regions where serum samples from sheep and goats were collected in Tanzania in 2013 and 2015.

Region	Year collected	Sheep	Goats	Total
Morogoro	2013	38	178	216
Shinyanga	2013	33	217	250
Pwani	2013	41	310	351
Simiyu	2013	59	202	261
Kagera	2013	12	198	210
Mwanza	2013	92	414	506
Dodoma	2013	180	420	600
Singida	2013	79	167	246
Tabora	2013	115	245	360
Katavi	2013	62	130	192
Kigoma	2013	64	136	200
Kilimanjaro	2015	66	80	146
Manyara	2015	55	92	147
Arusha	2015	56	97	153
Total		**952**	**2 886**	**3 838**

2.3.2 Detection of *peste des petits* ruminants antibodies using competitive enzyme-linked immunosorbent assay (cELISA)

Sera were tested for antibodies to PPRV using competitive ELISA (cELISA) (CIRAD EMVT, Montpellier, France). The test was performed according to manufacturer's instructions. Samples presenting a competition percentage of less than or equal to 50% were considered positive for PPRV antibodies.

2.3.3 Statistical analysis

Apparent prevalence estimates were used to estimate true prevalence (Rogan and Gladen, 1978) and the kit's relative diagnostic sensitivity and specificity of 92.2% and 98.9% respectively (Libeau *et al.*, 1995). The odds ratio was calculated to assess the association between being seropositive for PPR and animal species (Altman, 1991).

2.4 Results

From 3838 serum samples tested, 998 (26.0%) were positive; 758 (26.3%) of 2886 from goats and 240 (25.2%) of 952 from sheep (Table 2.2 and Figure 2.2). Overall true seroprevalence was 27.1% with 95% confidence interval ($CI_{95\%}$) of 25.6-28.5 and true seroprevalence for goats and sheep was 27.4% ($CI_{95\%}$: 25.7-29.1) and 26.2% ($CI_{95\%}$: 23.3-29.1), respectively. Morogoro region had the highest overall true seroprevalence at 72.8% ($CI_{95\%}$: 66.3-79.4) of antibodies against PPRV while Katavi region had the lowest at 2.1% ($CI_{95\%}$: -0.3-4.5). The odds of being seropositive to PPR was 1.06 ($CI_{95\%}$: 0.89-1.25) times higher in goats compared to sheep, a figure that is not statistically significant (Table 2.3).

Table 2.2: Regional seroprevalences of antibodies to *peste des petits* ruminants virus in goats and sheep in Tanzania in 2013 and 2015.

Region	Districts (Villages)	Goats			Sheep			Total		
		Total	Positive (%)	TP (95% CI)	Total	Positive (%)	TP (95% CI)	Total	Positive (%)	TP (95% CI)
Morogoro	2 (10)	178	124 (69.7)	73.5 (66.4 – 81.7)	38	25 (65.8)	69.4 (53.4 – 85.5)	216	149 (70.0)	72.8 (66.3 – 79.4)
Shinyanga	3 (4)	217	16 (7.4)	7.2 (3.5 – 10.9)	33	0	-	250	16 (6.4)	6.2 (2.9 – 9.4)
Pwani	3 (14)	310	90 (29.0)	30.3 (24/9 – 35.7)	41	13 (31.7)	33.1 (18 – 48.3)	351	103 (29.3)	30.6 (25.5 – 35.7)
Simiyu	3 (7)	202	15 (7.4)	7.3 (3.4 – 11.1)	59	6 (10.2)	10.2 (2 – 18.4)	261	21 (8.1)	7.9 (4.4 – 11.4)
Kagera	3 (16)	198	6 (3.0)	2.6 (0 – 5.1)	12	0	-	210	6 (2.9)	2.4 (0 – 4.8)
Mwanza	7 (23)	414	15 (3.6)	3.2 (1.3 – 5.1)	92	0	-	506	15 (3.0)	2.5 (0.9 – 4.1)
Kilimanjaro	1 (5)	80	29 (36.3)	38 (26.7 – 49.2)	66	12 (18.2)	18.7 (8.8 – 28.6)	146	41 (28.1)	29.3 (21.5 – 37)
Manyara	1 (5)	92	67 (72.8)	76.9 (67.2 – 86.6)	55	30 (54.5)	57.4 (43.4 – 71.5)	147	97 (66.0)	69.6 (61.5 – 77.8)
Arusha	1 (5)	97	66 (68.0)	71.8 (61.8 – 81.7)	56	37 (66.1)	69.7 (56.5 – 82.9)	153	103 (67.3)	71.1 (63.1 – 79)
Dodoma	3 (12)	420	239 (56.9)	60.2 (55.2 – 65.3)	180	104 (57.8)	60.9 (53.2 – 68.6)	600	344 (57.3)	60.4 (56.2 – 64.6)
Singida	2 (8)	167	39 (23.4)	24.2 (17.4 – 31.1)	79	0	-	246	39 (15.9)	16.2 (11.4 – 21.1)
Tabora	3 (3)	245	31 (12.7)	12.8 (8.4 – 17.3)	115	11 (9.6)	9.5 (3.8 – 15.3)	360	41 (11.4)	11.5 (8 – 15
Katavi	3 (3)	130	5 (3.8)	3.5 (-0.1 – 7)	62	0	-	192	5 (2.6)	2.1 (-0.3 – 4.5)
Kigoma	3 (3)	136	16 (11.8)	11.9 (6.1 – 17.7)	64	2 (3.1)	2.7 (-1.9 – 7.2)	200	18 (9.0)	8.9 (4.7 – 13.2)
Total	**38 (118)**	**2 886**	**758 (26.3)**	**27.4 (25.7 – 29.1)**	**952**	**240 (25.2)**	**26.2 (23.3 – 29.1)**	**3 838**	**998 (26.0)**	**27.1 (25.6 – 28.5)**

Total=total number of animals sampled, Positive=number of animals tested positive with percentage given in parenthesis, TP=true prevalence with 95% confidence interval in parenthesis, CI=confidence interval

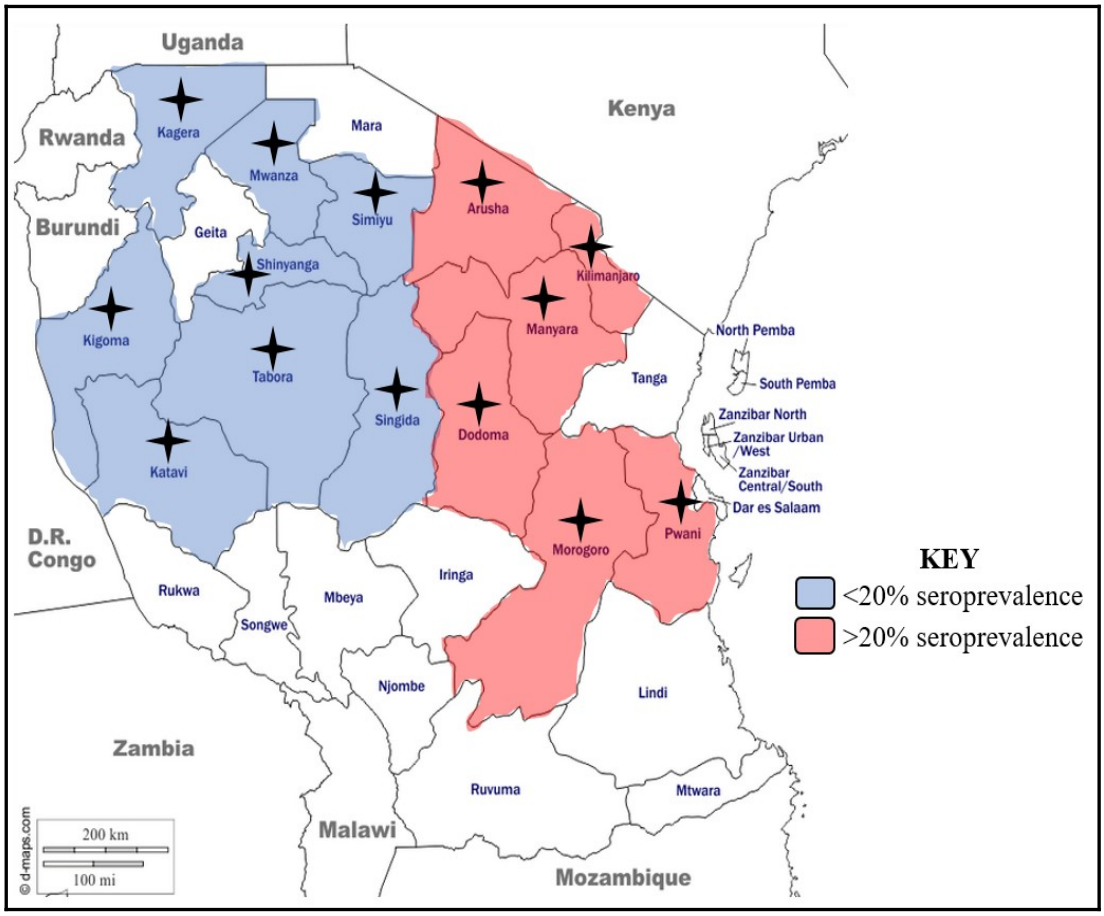

Figure 2.2: Map of Tanzania showing seroprevalences in study regions in 2013 and 2015.

Table 2.3: Association of species and being seropositive for *peste des petits* ruminants for samples collected in 2013 and 2015.

Variable	Odds ratio	95% CI	P value
Species (goats vs sheep)	1.06	0.89 - 1.25	0.507

2.5 Discussion

This study shows that PPR was widely prevalent in small ruminants in the study areas. All regions had seropositive cases. The overall observed true seroprevalence of 27.1% ($CI_{95\%}$: 25.6 - 28.5) (Table 2.2) is low compared to previous reports from northern and southern Tanzania performed in 2009 at 45.4% (Swai *et al.*, 2009) and 2012 at 31.0% (Muse *et al.*, 2012), respectively.

This difference may be attributed to vaccination campaigns carried out in 2008 (Arusha region) and 2010 (Morogoro and Mtwara regions) (URT, 2013), where animals are known to have lifelong immunity after vaccinations. Though the overall true seroprevalence is low, the figure is highly significant in a country with an estimated population of sheep around 8 million and goats around 16.7 million (URT, 2015).

Some regions registered true seroprevalence of less than 20% (Fig. 2.2), this indicates that PPR is widely prevalent in small ruminants in areas where the study was conducted. Data from studies in west, east and central Africa indicate that PPRV antibodies can be widespread among goats and sheep flocks raised in the tropics (Banyard *et al*., 2010; Megersa *et al.*, 2011; Abdalla *et al.*, 2012). Studies also indicate cELISA as a preferred diagnostic test for screening antibodies against the PPRV. This is because the test is simple, rapid, specific and sensitive for intensive surveillance (Singh *et al.,* 2004). Screening for antibodies against PPRV in different geographical areas of a country with varying climatic conditions has been helpful in developing disease control strategies (Balamurugan *et al*., 2011). Hence these results can be helpful to government officials in developing control strategies for Tanzania.The seropositivity difference between sheep and goats remains unclear in literature. In this study, prevalence between the two species was sheep 26.2% and goats 27.4%. The odds of being seropositive were 1.06 ($CI_{95\%}$: 0.89-1.25) in goats than in sheep, which implies there is no difference between the species.

This is in contradiction with some studies, including one carried out in Tanzania (Swai *et al*., 2009), which reported a higher seroprevalence in goats than in sheep and linked it to higher fecundity in goats compared to sheep (Rashid *et al.*, 2008; Zahur *et al*., 2011; Abdalla *et al*., 2012). Other studies have reported higher seroprevalence in sheep

than goats, attributing it to lower number of sheep sampled or due to the fact that goats are often affected more severely by the disease hence die prior to sampling (Khan *et al*., 2007; Megersa *et al.*, 2011). Therefore, more investigations are needed to further determine the variation between the species.

In 2008 and 2010, PPR vaccination was carried out around the 2008 outbreak foci (Arusha region) with another vaccination ring in Morogoro and Mtwara regions, shown in Figure 2.1 (MLFD, 2013). Arusha and Mtwara regions were chosen because they had already been seen as hotspot areas while Morogoro region acted as a buffer zone for spread of the disease from south (Mtwara region) to north. The use of antibiotics in managing clinical cases is also believed to increase survival rate of sick animals (Karimuribo *et al*., 2011) thus the surviving animals will carry antibodies to PPRV. These two factors may have contributed to the high seroprevalence because cELISA cannot discriminate from previously PPRV infected animals and vaccinated animals. Small ruminants vaccinated on a large scale with PPR vaccines will still test positive for antibodies to PPRV (Libeau, 2015). Despite this, presence of antibodies to PPRV in regions that previously were thought to be free and no vaccinations have been performed, such as Mwanza, Shinyanga, Kigoma and Tabora, indicates that the disease is spreading (Fig. 2.2). The data means future vaccinations should cover all regions of the country and not concentrate in known high risk areas. Small ruminants are easily moved especially for sale in markets. Live animal markets are an important vehicle for transmission of infectious diseases (Libeau, 2015). This was demonstrated in a study (Muse *et al.*, 2012) that found out that lack of appropriate veterinary services and inadequate infrastructure especially in the local animal markets in Tanzania may be facilitating transmission of PPR. Trade also brings about livestock theft that has been found to play a major role in

maintaining transmission of infectious diseases in many areas of East Africa (Megersa *et al*., 2011).

2.6 Conclusion

In conclusion, this study has confirmed the presence of PPR in regions of Tanzania that previously had little to no data on the disease. This is a step forward to getting information about the disease situation that can help to properly put into place systems and proper control measures to improve animal welfare and reduce episodes of disease outbreaks.

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2.8 Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

2.9 References

Abdalla, A. S., Majok, A. A., El Malik, K. H. and Ali, A. S. (2012). Sero-prevalence of *peste des petits* ruminants virus (PPRV) in small ruminants in Blue Nile, Gadaref and North Kordofan States of Sudan. *Journal of Public Health and Epidemiology* 4(3): 59 - 64.

Abraham, G., Sintayehu, A., Libeau, G., Albina, E., Roger, F., Laekemariam, Y., Abayneh, D. and Awoke, K. M. (2005). Antibody seroprevalences against *peste des petits* ruminants (PPR) virus in camels, cattle, goats and sheep in Ethiopia. *Preventive Veterinary Medicine* 70(1-2): 51 - 57.

Altman, D. G. (1991). *Practical Statistics for Medical Research.* Chapman and Hall, London.

Balamurugan, V., Saravanan, P., Sen, A., Rajak, K. K., Bhanuprakash, V., Krishnamoorthy, P. and Singh, R. K. (2011). Sero-epidemiological study of *peste des petits* ruminants in sheep and goats in India between 2003 and 2009. *Revue Scientifique et Technique de l`Office International des Epizooties* 30(3): 889 - 896.

Banyard, A. C., Parida, S., Batten, C., Oura, C., Kwiatek, O. and Libeau, G. (2010). Global distribution of *peste des petits* ruminants and prospects for improved diagnosis and control. *Journal of General Virology* 91(12): 2885 - 2897.

Gargadennec, L. and Lalanne, A. (1942). *La peste des petits ruminants* (The plague of small ruminants). *Bulletin des Services Zootechniques et des Epizooties*

del'Afrique Occidentale Francaise. *Bulletin of Zootechnical Services and Epizootics* 5: 16 - 21.

Geerts, S. (2009). Goat *Plague Or Peste Des Petits Ruminants*. *EAZWV Transmissible Disease Fact Sheet* 25. Institute of Tropical Medicine, Antwerp, Belgium.

Karimuribo, E. D., Loomu, P. M., Mellau, L. S. B. and Swai, E. S. (2011). Retrospective study on sero-epidemiology of *peste des petits* ruminants before its official confirmation in northern Tanzania in 2008. *Research Opinions in Animal and Veterinary Sciences* 1(3): 184 - 187.

Khan, H. A., Siddique, M., Arshad, M. J., Khan, Q. M. and Rehman, S. U. (2007). Seroprevalence of *peste des petits* ruminants virus in sheep and goats in Punjab province of Pakistan. *Pakistan Veterinary Journal* 27(3): 109 - 112.

Kivaria, F. M., Kwiatek, O., Kapaga, A. M., Geneviève, L., Mpelumbe-Ngeleja, C. A. R. and Tinuga, D. K. (2009). Serological and virological investigations on an emerging peste des petits ruminants virus infection in goats and sheep in Tanzania. In: *Proceedings of The 27th Tanzania Veterinary Association Scientific Conference*. 04 - 06 December 2009, Arusha, Tanzania.

Kivaria, F. M., Kwiatek, O., Kapaga, A. M., Swai, E. S., Libeau, G., Moshy, W., Mbyuzi, A. O. and Gladson, J. (2013). The incursion, persistence and spread of *peste des petits* ruminants in Tanzania: Epidemiological patterns and predictions. *Onderstepoort Journal of Veterinary Research* 80(1): 593.

Libeau, G., Prehaud, C., Lancelot, R., Colas, F., Guerre, L., Bishop, D. H. and Diallo, A. (1995). Development of a competitive ELISA for detecting antibodies to the *peste des petits* ruminants virus using a recombinant nucleoprotein. *Research in Veterinary Science* 58(1): 50 - 55.

Libeau, G., Diallo, A. and Parida, S. (2014). Evolutionary genetics underlying the spread of *peste des petits* ruminants virus. *Animal Frontiers* 4(1): 14 - 20.

Libeau, G. (2015). Current advances in serological diagnosis of *peste des petits* ruminants virus. In: Munir, M. (Eds) *Peste des petits* ruminants virus. Springer, Berlin, Heidelberg. pp. 133 - 154.

Megersa, B., Biffa, D., Belina, T., Debela, E., Regassa, A., Abunna, F., Rufael, T., Stubsjøen, S. M. and Skjerve, E. (2011). Serological investigation of *peste des petits* ruminants (PPR) in small ruminants managed under pastoral and agro-pastoral systems in Ethiopia. *Small Ruminant Research* 97(1-3): 134 - 138.

MLFD (2013). *Peste Des Petits Ruminants Progressive Control and Eradication Strategy*. Ministry of Livestock and Fisheries Development, Dar es Salaam.

Muse, E. A., Karimuribo, E. D., Gitao, G. C., Misinzo, G., Mellau, L. S. B., Msoffe P. L. M., Swai, E. S. and Albano, M. O. (2012). Epidemiological investigation into the introduction and factors for spread of *peste des petits* ruminants, southern Tanzania. *Onderstepoort Journal of Veterinary Research* 79(2): 457.

Rahman, A., Abubakar, M., Rasool, M. H., Manzoor, S., Saqalein, M., Rizwan, M., Munir, M., Ali, Q. and Wensman, J. J. (2016). Evaluation of risk factors for *peste des petits* ruminants virus in sheep and goats at the wildlife-livestock interface in Punjab Province, Pakistan. *BioMed Research International* 2016(2016): 7826245.

Rashid, A., Asim, M. and Hussain, A. (2008). Seroprevalence of *peste des petits* ruminants (PPR) virus in goats, sheep and cattle at livestock production research institute Bahadurnagar Okara. *The Journal of Animal and Plant Sciences* 18(4): 114 - 116.

Rogan, W. J. and Gladen, B. (1978). Estimating prevalence from the results of a screening test. *American Journal of Epidemiology* 107(1): 71 - 76.

Singh, R. P., Sreenivasa, B. P., Dhar, P., Shah, L. C. and Bandyopadhyay, S. K. (2004). Development of a monoclonal antibody based competitive-ELISA for detection and titration of antibodies to *peste des petits* ruminants virus. *Veterinary Microbiology* 98(1): 3 - 15.

Swai, E. S., Kapaga, A., Kivaria, F., Tinuga, D., Joshua, G. and Sanka, P. (2009). Prevalence and distribution of *peste des petits* ruminants virus antibodies in various districts of Tanzania. *Veterinary Research Communications* 33(8): 927 - 936.

URT (United Republic of Tanzania). (2013). *Peste des petits* ruminants progressive control and eradication strategy, Ministry of Livestock and Fisheries Development, Dar es Salaam, Government Printer.

URT (2015). *Tanzania Livestock Modernization Initiatives.* Ministry of Livestock and Fisheries Development, Dar es Salaam.

Wambura, P. N. (2000). Serological evidence of the absence of *peste des petits* ruminants in Tanzania. *Veterinary Record* 146(16): 473 - 474.

Zahur, A. B., Ullah, A., Hussain, M., Irshad, H., Hameed, A., Jahangir, M. and Farooq, M. S. (2011). Sero-epidemiology of *peste des petits* ruminants in Pakistan. *Preventive Veterinary Medicine* 102(1): 87 - 92.

CHAPTER THREE

3.0 DETECTION OF *PESTE DES PETITS* RUMINANTS AND CONCURRENT SECONDARY DISEASES IN SHEEP AND GOATS IN NGORONGORO DISTRICT, TANZANIA

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3.1 Abstract

Small ruminants play an important role in livelihoods of resource constrained communities. This study was initiated because of a massive outbreak of a respiratory disease in sheep and goats in Loliondo area in Ngorongoro district of Arusha region in Tanzania in 2016. During flock examination, a total of 240 serum samples and 61 nasal swabs were collected from clinically sick animals. Antibodies to *peste des petits* ruminants virus (PPRV), causative agent of *peste des petits* ruminants (PPR), were detected from sera using a competitive enzyme linked immunosorbent assay. A multiplex real-time reverse transcription polymerase chain reaction assay was used to detect four pathogens; PPRV, *Mycoplasma capricolum* subspecies *capripneumoniae*, *Pasteurella multocida* and *Capripoxvirus* from the nasal swabs. Overall seroprevalence of PPR was 74.6%, with all four pathogens detected from nasal swabs. Co-infections of PPRV and *Mycoplasma capricolum* subspecies *capripneumoniae*, PPRV and *Capripoxvirus*, PPRV and *Pasteurella multocida,* and *Mycoplasma capricolum* subspecies *capripneumoniae* and *Capripoxvirus* were also detected. Presence of PPR and the other diseases in this study provided insight into the severity of the outbreak in sheep and goats in Ngorongoro district. Thus, laboratory confirmation is critical for prompt and appropriate interventions to be made for control of diseases in sheep and goats with similar clinical signs. The findings also call for research into development of combined vaccines targeting common diseases of small ruminants in Tanzania.

Keywords: PPR; CCPP*;* goats; sheep

3.2 Introduction

Small ruminants contribute significantly to the economy of most rural communities in developing countries. Though small ruminants contribute towards alleviation of poverty, their productivity is hampered by, among other things, infectious diseases and poor husbandry practices (FAO and OIE, 2016). Compared with cattle, there are limited studies on small ruminants' health and the information available is fragmented and sometimes incomplete (Farougou *et al*., 2013). One area which is poorly documented is the magnitude of multiple infections, by different types of pathogens such as viruses, bacteria and parasites, in sheep and goats that result in respiratory diseases (Settypalli *et al*., 2016). Some clinical signs associated with respiratory diseases of small ruminants include ocular and nasal discharges, labored breathing, lesions in the oral and nasal mucus membranes, cough, pneumonia, diarrhea and severe dehydration (Roeder and Obi, 1999; Kul *et al*., 2015).

One important respiratory disease affecting small ruminants currently worldwide is *peste des petits* ruminants, PPR. *Peste des petits* ruminants is a viral disease of small ruminants first reported in West Africa in the early 1940s and later recognized as endemic in both West and Central Africa (Gargadennec and Lalanne, 1942; Parida *et al*., 2015). The disease is caused by *peste des petits* ruminants virus (PPRV), a member of the genus Morbillivirus of the *Paramyxoviridae* family, today re-classified as belonging to the species *Small ruminant morbillivirus,* genus *Morbillivirus* in the subfamily *Orthoparamyxovirinae* (ICTV, 2018). Currently, PPR is prevalent in Central, Eastern and Western Africa, Asia, and the Near and Middle East (Libeau *et al*., 2014). In East Africa, PPR was detected in Kenya and Uganda in 2007, while in Tanzania it was officially confirmed in 2008 (Kivaria *et al.,* 2009; Swai *et al*., 2009). The disease is considered

endemic in Tanzanian domestic sheep and goat populations (Torsson *et al*., 2017; Kgotlele *et al*., 2016).

Other respiratory diseases of economic importance affecting small ruminants include contagious caprine pleuropneumonia (CCPP), pasturella, sheep pox and goat pox (Brown *et al*., 1991; Ugochukwu and Agwu, 1991; Emikpe *et al*., 2010; Kul *et al*., 2015). *Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp) is the causative agent of CCPP, a highly contagious disease of goats first described in 1873 in Algeria (OIE, 2014). The disease was later described in 1976 in Kenya by MacOwan and Minette (1976), followed by subsequent isolations in several African countries including Tanzania (Bölske *et al*., 1995; Kusiluka *et al*., 2000a and 2000b). Sheep pox virus and goat pox virus, which belong to the *Capripoxvirus* genus, are responsible for pox diseases in sheep and goats. These occur in several parts of Africa, Asia, the Middle East and India (Spickler, 2015). *Pasteurella multocida* is also isolated from cases of respiratory diseases in sheep, goats, pigs and cattle, often causing pneumonia either alone or as an opportunist with other respiratory pathogens (Settypalli *et al*., 2016). Pneumonic pasteurellosis is one of the most economically important infectious diseases of ruminants with a wide prevalence throughout the continents (Mohammed and Abdelsalam, 2008). The similarity in the signs caused by these pathogens, and their co-localization in nearly the same endemic areas, call for appropriate differential diagnostic testing to accurately identify the responsible pathogen(s) (Settypalli *et al*., 2016). In this study, the aim was to investigate the etiological cause of a respiratory disease outbreak in sheep and goats in Loliondo area in Ngorongoro district of Arusha region in Tanzania.

3.3 Materials and methods

3.3.1 Study area

The study was conducted in Ngorongoro district, one of the districts of Arusha region in northern Tanzania in May 2016. The villages involved in the investigation were Sukenyan, Mondorosi, Ololosokwan and Enguserosambu (Fig. 3.1). The villages are inhabited by Maasai and Sonjo ethnic groups that are traditionally pastoralists and agro-pastoralists, respectively. The study area was chosen after a reported outbreak of a disease affecting sheep and goats with high mortality rates. The animals were said to present respiratory distress, diarrhoea and mucopurulent nasal discharges. The outbreak was reported to have been going on for four months before collection of samples for this study.

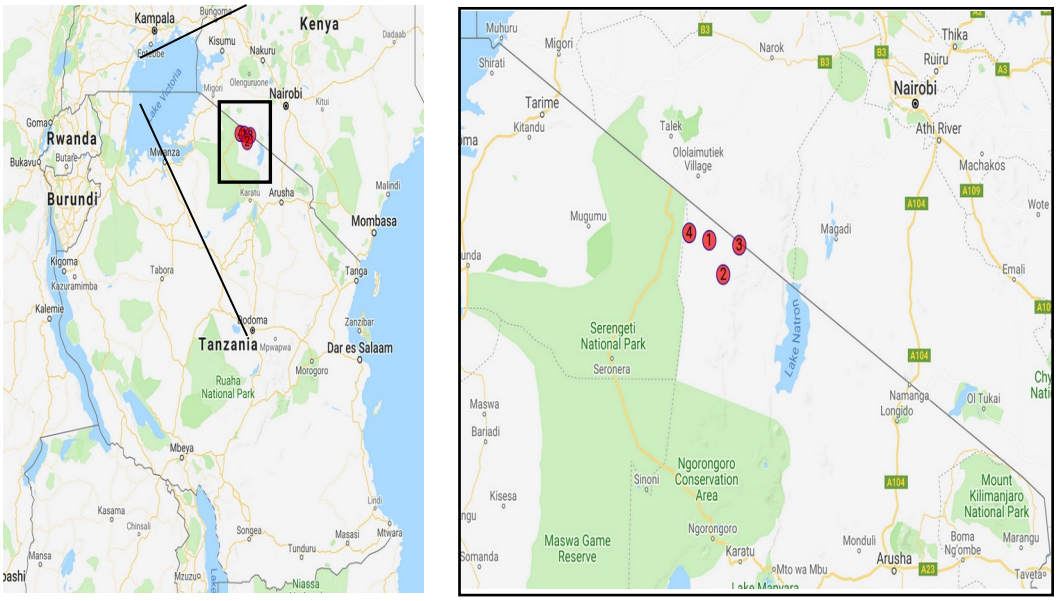

Figure 3.1: Map showing the study villages where samples were collected in Ngorongoro District. The numbers represent the villages, where; 1 is Mondorosi, 2 is Sukenyan, 3 is Enguserosambu and 4 is Ololosokwan.

3.3.2 Study animals and samples

Sheep and goats were randomly examined for clinical signs from the four villages. Clinical samples collected were 240 sera (from 59 sheep and 181 goats) and 61 nasal swabs (from 37 sheep and 24 goats) randomly from the four villages. Age and sex of sampled animals were recorded during sample collection.

3.3.3 Serological assay

Serum samples were tested for presence of antibodies to PPRV using ID screen® PPR competition ELISA (IDVet, Grabels, France). The kit was used and interpreted according to manufacturer's instructions.

3.3.4 Detection of nucleic acids

Total nucleic acids were extracted from nasal swabs using QIAamp Viral RNA Mini extraction kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The extracted samples were tested for the presence of nucleic acid material of four pathogens using a multiplex qRT-PCR method developed by Settypalli *et al.* (2016). Briefly, a 20 µL reaction volume containing reagents from iScript™ Universal Probes One-Step Kit (Bio-Rad, Hercules, USA), four pathogen-specific primer pairs (500 nM each) and probes (250 nM each) labeled at the 5'ends with different reporter dyes: *Capripoxvirus* with Cy5, PPRV with HEX, *P. multocida* with FAM and Mccp with Texas Red. The assay was run using CFX 96™ real-time PCR machine (Bio-Rad, Hercules, USA) with the following cycling conditions: 50°C for 20 mins followed by 95°C for 5 mins and 40 cycles of denaturation at 94°C for 10 secs, annealing at 56°C for 20 secs, and extension at 62°C for 20 secs. The data acquisition was performed during the annealing step. An amplification peak and quantification cycle (Cq) value of less than 35 was interpreted to indicate presence of a pathogen.

3.3.5 Data analysis

Data generated were entered in Microsoft Excel 2016 and analyzed using descriptive statistics. Odds ratio (OR) was calculated according to Altman (1991) using serological data. The OR assesses the association of being seropositive for PPR where p-value < 0.05 was considered as significant.

3.4 Results

3.4.1 General observations of animals

Different clinical signs were observed from sheep and goats examined randomly in the four villages. Generally, most clinical signs observed were suggestive of different diseases. Clinical signs observed in the examined flocks included nodules on skin of some animals, nasal discharges, loss of body condition and diarrhoea. A combination of nasal discharges and diarrhoea was the most prevalent clinical sign in all herds examined with some diarrhoea tinged with blood. Figure 3.2 below shows some of the animals with nasal discharges and diarrhoea.

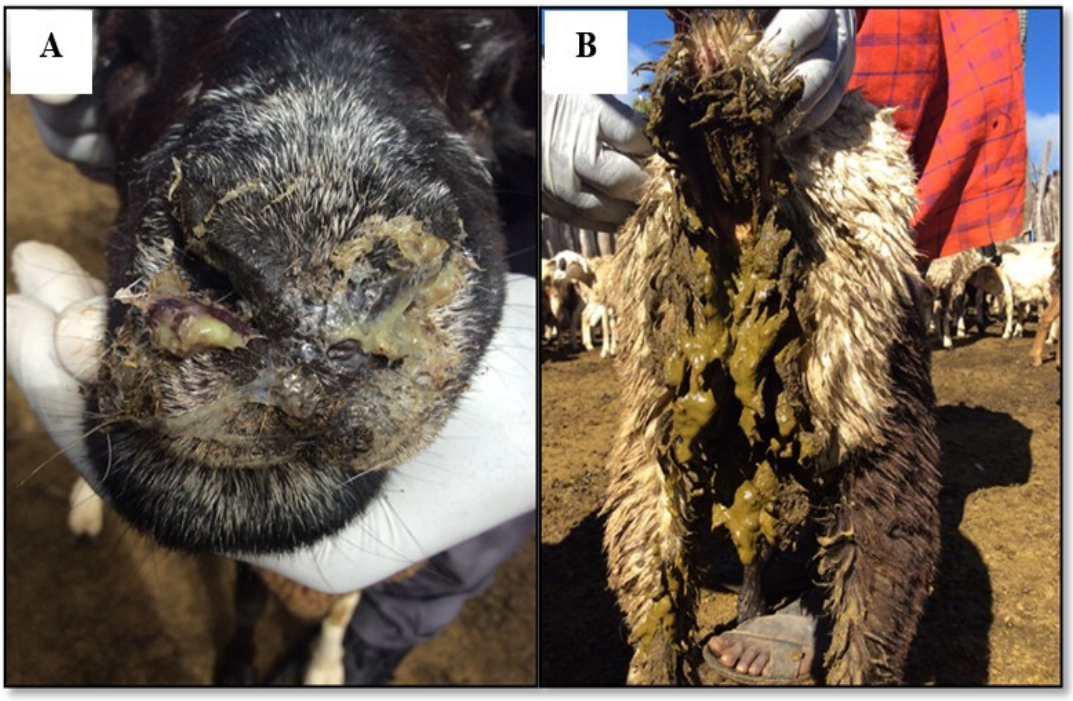

Figure 3.2: Clinical signs observed in goats in Ngorongoro district. A goat with mucopurulent nasal discharge (A) and a goat with diarrhoea (B).

3.4.2 Serological examination

Serological examination of samples collected from sheep and goats indicates PPR occurrence with an overall seroprevalence of antibodies to PPRV at 74.6%. There was no statistical difference of seropositivity between species, sheep and goats, and in sex, being male or female in this study as indicated by p-values > 0.05 (Table 3.1). Significant difference ($p < 0.05$) was noted in age where animals less than 2 years were less likely to be seropositive for antibodies to PPRV than animals older than 2 years.

Table 3.1: Seroprevalences of antibodies to PPRV in goats and sheep in Ngorongoro district, Tanzania.

Variable	Category	Total	Positives (%)	OR (95%CI)	p-value
Species	Goat	181	137 (75.7)	1.26 (0.65-2.43)	0.490

	Sheep	59	42 (71.2)		
Age	≤ 2 years	84	53 (63.1)	0.329 (0.19-0.6)	0.0002
	> 2 years	156	126 (80.8)		
Sex	Female	167	126 (75.4)	1.16 (0.62-2.16)	0.641
	Male	73	53 (72.6)		

3.4.3 Multiplex qRT-PCR analysis

Of the 61 nasal swabs tested, 38 were positive for one or more of the four pathogens being analyzed (Table 3.2). Majority of the pathogens were detected from nasal swabs collected in goats than in sheep. The most detected pathogen was *P. multocida* while the least detected pathogen was Mccp. Co-infection of Mccp/CaPV was also observed to be common in nasal swabs from goats than in sheep.

Table 3.2: Detected pathogens that cause similar respiratory syndromes in sheep and goats in Ngorongoro district using one-step multiplex qRT-PCR test.

Pathogen(s) detected	**Sheep (%) n=37**	**Goats (%) n=24**	**Total (%) n=61**
PPRV[a]	2 (5)	3 (13)	5 (8)
Mccp[b]	1 (3)	2 (8)	3 (5)
P. multocida[c]	12 (32)	7 (29)	19 (31)
CaPV[d]	2 (5)	2 (8)	4 (7)
PPRV/Mccp	1 (3)	0	1 (2)
PPRV/CaPV	1 (3)	0	1 (2)
PPRV/*P. multocida*	1 (3)	0	1 (2)
Mccp/CaPV	1 (3)	3 (13)	4 (7)
Total positive	**21 (57)**	**17 (71)**	**38 (62)**

[a]*peste des petits* ruminants virus, [b]*Mycoplasma capricolum* subspecies *capripneumoniae*, [c]*Pasteurella multocida* and [d]*Capripoxvirus*

3.5 Discussion

Clinical signs observed in the examined animals were nasal discharges and diarrhoea. These clinical signs are expressed in many infectious diseases of small ruminants.

Infectious diseases that present similar observed clinical signs include; PPR, pneumonic pasteurellosis and CCPP for nasal discharges; PPR, coccidiosis or gastro-intestinal helminth infestations for diarrhea; and contagious ecthyma, sheep pox and goat pox for nodular lesions (Roeder and Obi, 1999; Diallo *et al.*, 2007; Munir *et al*., 2009; Zro *et al*., 2014). In Tanzania, difficulty in differentiating clinical signs in sheep and goats has been mentioned as a major limiting factor in diagnosis especially for PPR and CCPP (Mbyuzi *et al*., 2014). In such cases where specific manifestations of the diseases are absent, reliable laboratory tests are needed.

The overall seroprevalence of PPR in this study was 74.6%. This seroprevalence is in line with findings from a recent study that analyzed samples from 14 regions of Tanzania, with Arusha region having an overall seroprevalence of 71.1% (Kgotlele *et al*., 2016). Other studies done in northern Tanzania have found PPR seroprevalence of 45.5% in 2008 (Swai *et al*., 2009) and 22.1% in 2008-09 (Kivaria *et al*., 2013). This variation over the years may be due to uncontrolled animal movement that spread PPR in the sheep and goat population reared without proper vaccination. In this study, presence of antibodies to PPRV was most likely caused by natural exposure as none of the animals were vaccinated according to records from the District Veterinary Office. The animals in this study were grazed in communal pastures, which could be where they encounter infected animals. Intermingling of animals in communal grazing lands has been found to facilitate spread of infectious diseases such as PPR, CCPP, contagious ecthyma, goat and sheep-pox (Kusiluka and Kambarage, 1996).

There was statistical difference between animals under 2 years less likely to be seropositive than those above 2 years. Studies have shown that differences noted between the age groups are most likely due to younger animals loosing their aquired passive

immunity after three months and the older ones having life long immunity after survival of infection (Kul *et al*., 2015). In this study, there was no statistical difference between males and females. This finding is different from another study done in Tanzania where females were more likely to be seropositive than males (Torsson *et al*., 2017). It is believed that females are used in reproduction hence kept longer in the herd than males, therefore have a longer risk period for exposure in the herd. Stress associated with pregnancy and milk production could also predispose them to infection (Aziz-Ul *et al*., 2016).

All four pathogens investigated in this study were detected from nasal swabs, including co-infections of PPRV and Mccp, PPRV and CaPV, PPRV and *P. multocida*, and Mccp and CaPV. Co-association of PPRV with other viral and bacterial infections in small ruminants have been demonstrated before in other studies in different parts of the world (Kul *et al*., 2015). Viral diseases with co-association with PPRV include sheep and goat pox virus, while co-associations with bacterial pathogens include *Pasteurella* spp., Mccp and *Mannheimia haemolytica* (Brown *et al*., 1991; Ugochukwu and Agwu 1991; Emikpe *et al*., 2010; Malik *et al*., 2011). The co-infections detected in this study may have provided complications during field diagnosis that resulted in the persistence and severity in the affected flock. Thus, laboratory confirmation is critical for appropriate interventions to be made for the different diseases of sheep and goats especially regarding PPR. Development and use of specific diagnostic tests that can distinguish PPR from diseases with similar signs has helped unquestionably to improve knowledge and understanding in geographical distribution and spread of the disease in specific areas (Libeau, 2015). This is critical as PPR has been identified as the next animal disease to be eradicated after rinderpest. One of the factors identified in the PPR Global Control and Eradication Strategy (PPR GCES) that make eradication possible is availability of

appropriate diagnostic tests and protocols for surveillance (FAO and OIE 2016). Thus, help attain one of the objectives to progressively reduce the incidence and spread of PPR and ultimately eradicate.

3.6 Conclusion

In conclusion, PPRV, Mccp, CaPV and *P. multocida* were detected in sheep and goats in Ngorongoro district. The study also confirmed occurrence of co-infection of pathogens that are associated with respiratory distress in sheep and goats in Tanzania, an important factor for consideration in small ruminant disease diagnosis and control strategies especially for PPR.

3.7 Acknowledgements

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3.8 References

Altman, D. G. (1991). *Practical Statistics for Medical Research*. Chapman and Hall, London.

Aziz-Ul-Rahman, Abubakar, M., Rasool, M. H., Manzoor, S., Saqalein, M., Rizwan, M., Munir, M., Ali, Q. and Wensman, J. J. (2016). Evaluation of risk factors for *peste des petits* ruminants virus in sheep and goats at the wildlife-livestock interface in Punjab Province, Pakistan. *BioMed Research International* 2016(2016): 7826245.

Bölske, G., Johansson, K. E., Heinonen, R., Panvuga, P. A. and Twinamasiko, E. (1995). Contagious caprine pleuropneumonia in Uganda and isolation of *Mycoplasma*

capricolum subsp. *capripneumoniae* from goats and sheep. *The Veterinary Record* 137(23): 594.

Brown, C. C., Mariner, J. C. and Olander, H. J. (1991). An immunohistochemical study of the pneumonia caused by *peste des petits* ruminants virus. *Veterinary Pathology* 28(2): 166 - 170.

Diallo, A., Minet, C., le Graff, C., Berhe, G., Albina, E., Libeau, G. and Barrett, T. (2007). The threat of *peste des petits* ruminants progress in vaccine development for disease control. *Vaccine* 25(30): 5591 - 5597.

Emikpe, B. O., Sabri, M. Y., Akpavie, S. O. and Zamri-Saad, M. (2010). Experimental infection of *peste des petits* ruminants virus and Mannheimia haemolytica A2 in goats: immunolocalisation of Mannheimia haemolytica antigens. *Veterinary Research Communications* 34(7): 569 - 578.

FAO and OIE (2016). *Peste Des Petits Ruminants Global Eradication Programme; Contributing to Food Security, Poverty Alleviation and Resilience - Five Years (2017–2021*). Food and Agriculture Organization of the United Nations and the World Organisation for Animal Health, Rome. 62pp.

Farougou, S., Gagara, M. and Mensah, G. A. (2013). Prevalence of *peste des petits* ruminants in the arid zone in the Republic of Niger. *Onderstepoort Journal of Veterinary Research* 80(1): 544.

Gargadennec, L. and Lalanne, A. (1942). *La peste des petits ruminants* (The plague of small ruminants). *Bulletin des Services Zootechniques et des Epizooties del'Afrique Occidentale Francaise*. *Bulletin of Zootechnical Services and Epizootics* 5: 16 - 21.

ICTV International Committee on Taxonomy of Viruses (2018). [https://talk.ictvonline.org/taxonomy/] site visited on 5/2/2018.

Kgotlele, T., Torsson, E., Kasanga, C. J., Wensman, J. J., and Misinzo, G. (2016). Seroprevalence of *peste des petits* ruminants virus from samples collected in different regions of Tanzania in 2013 and 2015. *Journal of Veterinary Science and Technology* 7: 394.

Kivaria, F. M., Kwiatek, O., Kapaga, A. M., Geneviève, L., Mpelumbe-Ngeleja, C. A. R. and Tinuga, D. K. (2009). Serological and virological investigations on an emerging peste des petits ruminants virus infection in goats and sheep in Tanzania. In: *Proceedings of The 27th Tanzania Veterinary Association Scientific Conference*. 04 - 06 December 2009, Arusha, Tanzania.

Kivaria, F. M., Kwiatek, O., Kapaga, A. M., Swai, E. S., Libeau, G., Moshy, W., Mbyuzi, A. O. and Gladson, J. (2013). The incursion, persistence and spread of *peste des petits* ruminants in Tanzania: Epidemiological patterns and predictions. *Onderstepoort Journal of Veterinary Research* 80(1): 593.

Kul, O., Atmaca, H. T. and Munir, M. (2015). Pathology of *peste des petits* ruminants virus infection in small ruminants and concurrent infections. In: Munir, M. (Ed) *Peste des Petits Ruminants Virus.* Springer-Verlag Berlin Heidelberg. pp. 119 - 131.

Kusiluka, L. J. M., Ojeniyi, B., Friis, N. F., Kazwala, R. R. and Kokotovic, B. (2000a). Mycoplasmas isolated from the respiratory tract of cattle and goats in Tanzania. *Acta Veterinaria Scandinavica* 41(3): 299 - 309.

Kusiluka, L. J. M., Semuguruka, W. D., Kazwala, R. R., Ojeniyi, B. and Friis, N. F. (2000b). Demonstration of Mycoplasma capricolum subsp. capripneumoniae and Mycoplasma mycoides subsp. mycoides small colony type in outbreaks of caprine pleuropneumonia in eastern Tanzania. *Acta Veterinaria Scandinavica* 41(3): 311 - 319.

Kusiluka, L. and Kambarage, D. (1996). Diseases of small ruminants in sub-Saharan Africa: A handbook common diseases of sheep and goats in sub-Saharan Africa. VETAID, ISBN 09522299 5, 116pp.

Libeau, G. (2015). Current advances in serological diagnosis of *peste des petits* ruminants virus. In: Munir, M. (Ed) *Peste des Petits Ruminants Virus.* Springer, Berlin, Heidelberg. pp. 133 - 154.

Libeau, G., Diallo, A. and Parida, S. (2014). Evolutionary genetics underlying the spread of *peste des petits* ruminants virus. *Animal Frontiers* 4(1): 14 - 20.

MacOwan, K. J. and Minette, J. E. (1976). A mycoplasma from acute contagious caprine pleuropneumonia in Kenya. *Tropical Animal Health and Production* 8(2): 91 - 95.

Malik, Y. P. S., Singh, D., Chandrashekar, K. M., Shukla, S., Sharma, K., Vaid, N. and Chakravarti, S. (2011). Occurrence of dual infection of *peste des petits* ruminants and goatpox in indigenous goats of central India. *Transboundary and Emerging Diseases* 58(3): 268 - 273.

Mbyuzi, A. O., Komba, E. V., Kimera, S. I. and Kambarage, D. M. (2014). Sero-prevalence and associated risk factors of *peste des petits* ruminants and contagious caprine pleuro-pneumonia in goats and sheep in the Southern Zone of Tanzania. *Preventive Veterinary Medicine* 116(1-2): 138 - 144.

Mohammed, R. A. and Abdelsalam, E. B. (2008). A review on pneumonic pasteurellosis (respiratory mannheimiosis) with emphasis on pathogens' virulence mechanisms and predisposing factors. *Bulgaria Journal of Veterinary Medicine* 11(3): 139 - 160.

Munir, M., Abubakar, M., Khan, M. T. and Abro, S. H. (2009). Comparative efficacy of single radial haemolysis test and countercurrent immune electro-osmophoresis with monoclonal antibodies based competitive ELISA for the serology of *peste*

des petits ruminants in sheep and goats. *Bulgaria Journal of Veterinary Medicine* 12(4): 246 - 253.

OIE (2014). Contagious *Caprine Pleuropneumonia. In: Chapter 2.7.6. Manual of Diagnostic Tests And Vaccines For Terrestrial Animals*. OIE, Paris, 15pp.

Parida, S., Muniraju, M., Mahapatra, M., Muthuchelvan, D., Buczkowski, H. and Banyard, A. C. (2015). *Peste des petits* ruminants. *Veterinary Microbiology* 181(1-2): 90 - 106.

Roeder, P. and Obi, T. (1999). *Recognizing PPR A Field Manual*. Food and Agriculture organization room. pp. 175 - 181.

Settypalli, T. B. K., Lamien, C. E., Spergser, J., Lelenta, M., Wade, A., Gelaye, E., Loitsch, A., Minoungou, G., Thiaucourt, F. and Diallo, A. (2016). One-step multiplex RT-qPCR assay for the detection of *peste des petits* ruminants virus, *Capripoxvirus*, *Pasteurella multocida* and *Mycoplasma capricolum* subspecies (ssp.) *capripneumoniae*. *PLoS One* 11(4): e0153688.

Spickler, A. R. (2015). *Peste des petits* ruminants http://www.cfsph. iastate.edu/ DiseaseInfo/factsheets.php site visited on 15/02/2018.

Swai, E. S., Kapaga, A., Kivaria, F., Tinuga, D., Joshua, G. and Sanka, P. (2009). Prevalence and distribution of *peste des petits* ruminants virus antibodies in various districts of Tanzania. *Veterinary Research Communications* 33(8): 927 - 936.

Torsson, E., Berg, M., Misinzo, G., Herbe, I., Kgotlele, T., Päärni, M., Roos, N., Blomström, A., Ståhl, K. and Wensman, J. J. **(**2017). Seroprevalence and risk factors for *peste des petits* ruminants and selected differential diagnosis in sheep and goats in Tanzania. *Infection Ecology and Epidemiology* 7: 1368336.

Ugochukwu, E. I. and Agwu, C. O. (1991). Aerobic bacteria from nasal discharge of goats suffering from clinical PPR: Isolation and identification. *Microbios* 65(263): 81 - 85.

Zro, K., Zakham, F., Melloul, M., Fahime, E. E. and Ennaji, M. M. (2014). A sheeppox outbreak in Morocco: Isolation and identification of virus responsible for the new clinical form of disease. *BMC Veterinary Research* 10: 31.

CHAPTER FOUR

4.0 MOLECULAR CHARACTERIZATION OF *PESTE DES PETITS* RUMINANTS VIRUS IN A FARM IN MELELA MLANDIZI VILLAGE IN TANZANIA FROM 2014 TO 2016

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Manuscript

4.1 Abstract

Peste des petits ruminants is an acute viral disease of small ruminants caused by p*este des petits* ruminants virus, PPRV. The disease is found in almost 70 countries in Africa, the Middle East and parts of Asia. The disease is endemic in Tanzania, with the first cases reported in 2008 in the Northern Zone districts bordering Kenya. This study describes presence of the disease in a traditional pastoral farm in Melela Mlandizi village in Morogoro region from 2014 to 2016. A total of 86 samples (sera, swabs, whole blood and tissues) were collected. Observations in animals in all the years of study only showed nasal discharges. Antibodies to PPRV were detected in samples collected in all the years. The virus was detected in samples collected in 2014 and 2015 while no virus was detected in samples collected in 2016. Two positive samples in 2015 were characterized as belonging to lineages II and III. This is the second time lineage II is reported in Tanzania, reaffirming its presence in the Tanzanian herd. In conclusion, presence of PPR was confirmed in the farm in Melela village over the years of study even though the animals did not have typical clinical signs associated with PPR. Presence of two lineages were confirmed from animals in the same herd, another complex situation to be considered and further studied in the virus epidemiology in Tanzania.

4.2 Introduction

Peste des petits ruminants (PPR) is a highly infectious disease of small domestic ruminants. The disease is currently considered one of the main transboundary animal diseases that constitute a threat to small ruminant production in many developing countries (Banyard *et al.*, 2010). The disease is caused by *peste des petits* ruminants virus (PPRV), a member of the genus *Morbillivirus* of the *Paramyxoviridae* family (Kwiatek *et al.*, 2010). Though the virus is serologically monotypic, there are four genetic lineages (I, II, III and IV) circulating globally (Banyard *et al.,* 2010; Kwiatek *et al.,* 2011; Adombi *et al*., 2017). The lineages are based on partial sequences of the nucleoprotein or fusion protein. Lineages I, II and III were first found in Africa, while lineage IV was found in Asia including the Middle and Near East (Banyard *et al*., 2010; Kwiatek *et al.,* 2011). Currently, lineages II, III and IV are actively circulating, while lineage I was last reported in Senegal in 1994 (Diop *et al.,* 2005; Banyard *et al.*, 2010; Dundon *et al*., 2014; Libeau *et al.,* 2014).

In Tanzania, PPR was first reported in 2008 in the Northern Zone districts bordering Kenya (Karimuribo *et al*., 2011). The disease was later introduced into southern Tanzania in 2009 through purchased goats from a livestock market located about 700 km in the outskirts of Dar es Salaam City (Muse *et al*., 2012). By the end of 2012, the disease had further spread to eastern and southern parts of the country affecting about 28 districts (URT, 2013). Lineages currently identified in Tanzania are lineages II and IV in southern Tanzania and lineage III in northern and eastern Tanzania (Kgotlele *et al*., 2014; Misinzo *et al.,* 2015). All these were identified during confirmed outbreaks of PPR with animals showing typical clinical manifestations of the disease. This report describes presence of PPR in a farm from November 2014 to March 2106.

4.3 Materials and methods

4.3.1 Study area and sample collection

The study was carried out in Melela Mlandizi village, Mvomero district, Morogoro region (Fig. 4.1). Morogoro climate varies from warm tropical to cool, with a short rainy season in October-December and a long rainy season in February-May, with crop farming, agro-pastoralist and pastoralist as the major farming systems (URT, 2012). The study area was purposively targeted due to past incidences of PPR outbreaks in Mvomero district (Kgotlele *et al*., 2014; Namtimba, 2015). Samples were collected in November 2014, March 2015 and March 2016 from the same farm. The farm is located along the Zambia highway where cattle, sheep, goats and chickens are kept. A small patch of land for growing maize is also found in the farm to feed both the people and animals. The farm had 28 animals (goats and sheep) the first year (2014). There were no deaths and no new animals introduced during the study years except two new births in 2016.

Sample size was calculated as follows (Daniel, 1999):

$$n = N*X / (X + N - 1) \quad \ldots\ldots(1)$$

Where:

$X = Z_{\alpha/2}^2 * p*(1-p) / MOE^2$

$Z_{\alpha/2}$ is the critical value of the Normal distribution at $\alpha/2$

(at confidence level of 95%, α is 0.05 and the critical value is 1.96)

MOE is the margin of error = 5%

p is the sample proportion = 50%

N is the population size = 28

From the formula, calculated sample size is 27 samples per year (81 samples for three years). A total of 86 samples were collected instead of 81 due to the small number of animals found in the farm. Samples collected were; 28 in 2014, 28 in 2015 and 30 in 2016. The samples collected were sera, nasal and/or ocular swabs, whole blood and tissues (lungs and intestines) from sheep and goats. Tissues were collected from sacrificed animals.

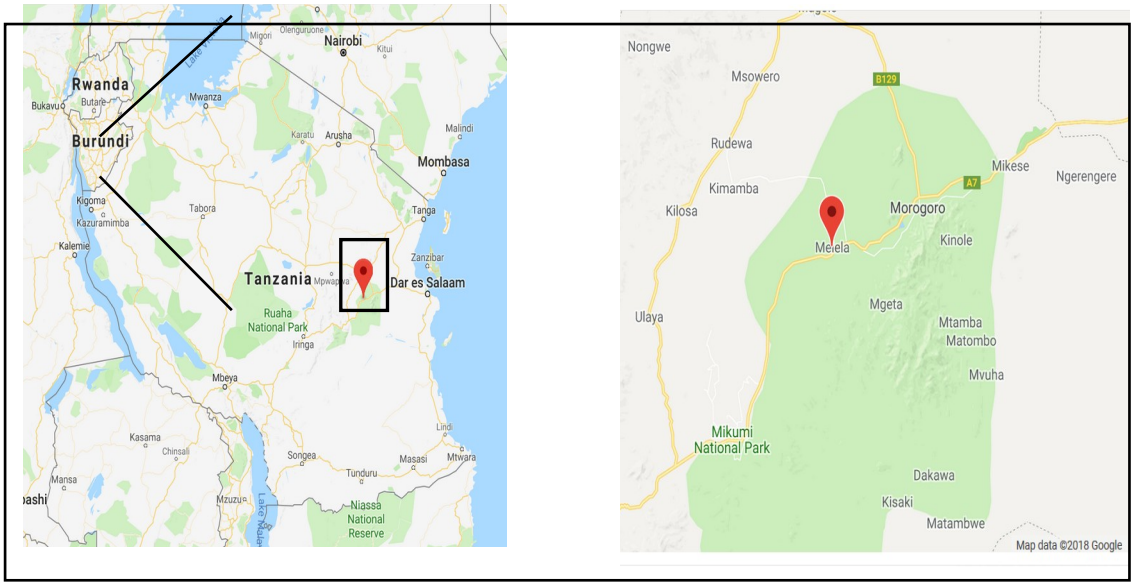

Figure 4.1: Map showing location of the farm in Melela Mlandizi village where samples were collected in 2014, 2015 and 2016. The farm is located south of Morogoro town.

4.3.2 Sample preparation and laboratory analysis

Sera samples were tested for presence of antibodies to PPRV using the ID screen® PPR competition ELISA (ID.Vet, Grabels, France). The kit was used and interpreted according to manufacturers' instructions. Briefly, 25 µL of samples and controls were added to the antigen coated wells and incubated for 45 mins at 37°C. The plates were washed three times with washing buffer. Then, 100 µL of anti-PPRV nucleoprotein horseradish

peroxidase conjugate was applied to wells and again incubated for 30 mins to cover unbound antigens.

Washing was done again three times followed by adding 100 µL of substrate solution and incubated for 15 mins. The reaction was stopped with 100 µL stop solution and reading was taken at 450nm on ELISA reader (Multiskan Ex, Thermo Corp, USA).

Viral RNA was extracted from whole blood, tissue suspension and swabs using QIAamp Viral RNA Mini extraction kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Briefly, samples were lysed using a lysis buffer followed by protein precipitation using ethanol. The lysate was then passed through a Qiagen column. The bound RNA was washed with the buffers and finally eluted with RNase free water.

Extracted RNA was amplified in a 25 µL reaction using AgPath-ID One-Step RT-PCR kit (Applied Biosystems, Courtaboeuf, France) and PPRV specific primers: NP3 (5'-TCTC GGAA ATCG CCTC ACAG ACTG-3') and NP4 (5'-CCTC CTCC TGGT CCTC CAGA ATCT-3') from Couacy-Hymann *et al*. (2002). Amplification was carried out in GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA) followed by electrophoresis on a 1.5% agarose gel. The DNA was visualized by ultraviolet fluorescence with positive samples showing 351bp bands. Samples that tested positive for the nucleoprotein were sequenced using the Sanger sequencing method and separated on a genetic analyzer ABI 3730 (Applied Biosytems, California, USA).

4.3.3 Bioinformatics analysis

The sequences generated were edited and assembled in BioEdit version 7.1.7 (Hall, 1999). Homologous nucleotide sequences were searched and retrieved from the NCBI GenBank using Basic Local Alignment Search Tool (BLAST®). Alignment and

construction of the phylogenetic tree were done in MEGA 5.05® (CEMI, Tempe, AZ, USA) employing ClustalW algorithm and neighbour-joining method with 1000 bootstrap replicates, respectively (Tamura *et al.*, 2011).

4.4 Results

The only clinical sign observed in all the years was nasal discharges (Fig. 4.2). This was observed in goats (males and females) and in both the young and old animals, while sheep did not have any clinical signs.

Figure 4.2: Some of the animals that were sampled in Melela Mlandizi village showing nasal discharges in 2014 (A) and in 2016 (B).

A total of 86 samples (45 sera, 2 tissues, 11 whole blood and 28 swabs) were collected from both goats and sheep in all the three years of this study. Figure 4.3 shows the overall trend of the results for the years 2014, 2015 and 2016. Overall, there was a decline in presence of the virus and antibodies to PPRV from 2014 to 2016. In 2016, only seropositive animals were found and no PCR positive animals.

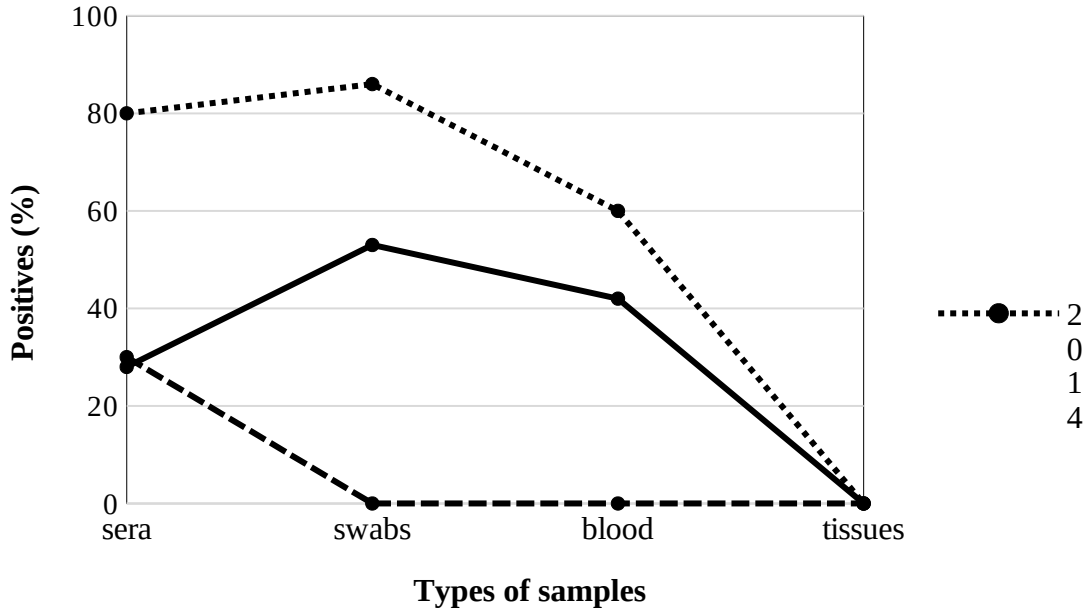

Figure 4.3: Trend analysis of PPR positive samples collected in 2014, 2015 and 2016 in a farm in Melela Mlandizi village. Positivity here means antibody-positive in sera and PCR-positive in swabs, blood and tissues.

Phylogenetic analysis of the partial nucleoprotein gene was performed with two representative sequences from two samples collected in 2015 (TAN/MelelaG/2015 from a goat and TAN/MelelaS/2015 from a sheep) and sequences representing all four lineages using MEGA5 software. The analysis revealed that the sequences were clustered in two distinct lineages, II and III (Fig. 4.4).

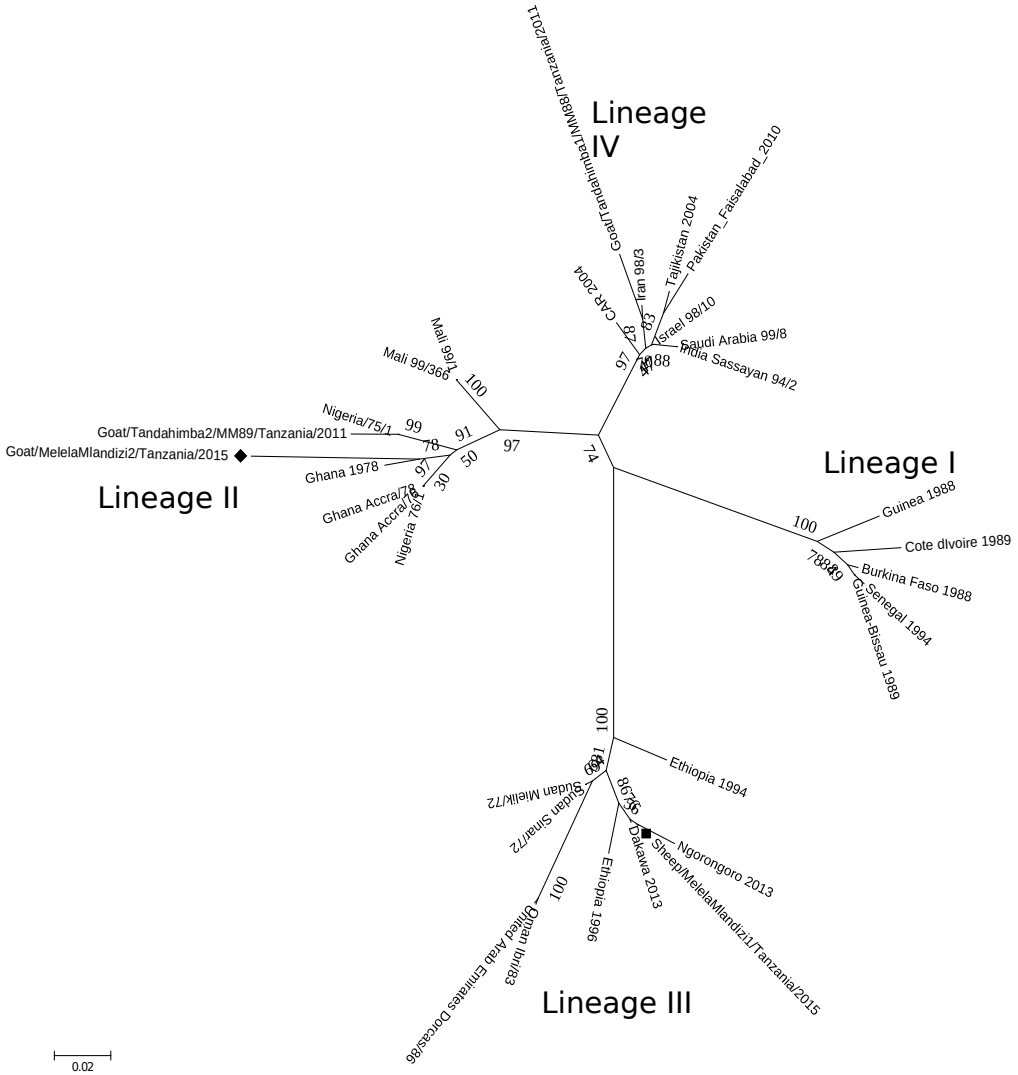

Figure 4.4: Phylogenetic analysis of partial nucleoprotein gene of PPRV samples collected in Melela Mlandizi village constructed using the neighbour-joining method in MEGA 5.05® software. Samples from this study are marked with a black diamond shape and values along the branches indicate bootstrap values of 1000 replicates.

4.5 Discussion

Presence of PPR in sheep and goats in this farm is of interest because it is found along the highway to Zambia and southern Africa. Hence, it can be a possible route of transmission in the spread of PPR to the rest of the southern African countries. Only goats exhibited excess nasal discharges but did not show any other clinical presentations typical of PPR in all the years of sampling. This can be attributed to the type of breed found in the district which is mainly indigenous. Clinical signs can be affected by type of breed of sheep and goats as some breeds fail to develop characteristic clinical signs especially indigenous goats and sheep (Munir *et al*., 2012). Failure of some breeds to exhibit clinical signs can lead to spread of the virus as animals would be mistaken to be apparently healthy hence can either be sold or transported. However, presence of nasal discharges alone cannot be used to diagnose PPR as there are other infectious diseases such as pneumonic pasteurellosis and CCPP that present nasal discharges (Roeder and Obi, 1999; Diallo *et al.,* 2007; Munir *et al*., 2009; Zro *et al*., 2014).

Using a cELISA test, antibodies to PPRV were detected in all the years of sampling. The level of antibodies declined in 2015 when compared with 2014 but remained the same for 2015 and 2016 (Fig. 4.3). At the time of sampling, the farm had no history of PPR vaccination suggesting that the antibodies were due to an infection of PPR at one time. Presence of antibodies whether to a wild virus or a vaccine is a marker of the host immune protection against infection, in this case PPR (Libeau, 2015). Using conventional PCR for detection of the nucleoprotein, PPRV was isolated from samples collected in 2014 and 2015 in both blood and swabs. No virus was isolated from samples collected in 2016. Plausible explanation is the flock may have come into contact with an infected flock to illicit production of antibodies without necessarily contracting the virus. Similar

findings were observed by Kivaria *et al*. (2013), citing presence of these ‘silent spreaders’ coupled with uncontrolled livestock movement within the country and across international borders as a reason why antibodies maybe detected without presence of the clinical signs.

Using both serology and PCR results from all the years in this study, it is apparent that presence of antibodies in the herd in 2016 was from animals that survived an infection as there was no active virus isolated. Animals that survive infection develop antibodies to PPRV usually from seven to ten days’ post infection which then persist in the long term (Diop *et al.,* 2005). Studies of outbreaks of PPR in India have also suggested that a larger population of animals get affected in the first outbreak, but in subsequent outbreaks, only few animals get affected due to presence of protective level of antibodies (Balamurugan *et al.,* 2015). The declining and stabilizing of antibodies is also reported to be a characteristic in an endemic situation such as Tanzania (Libeau, 2015). Hence, understanding transmission dynamics under different production systems in an endemic setting is important for control purposes.

The two sequenced samples from this study were clustered within lineage II (Goat/MelelaMlandizi/Tanzania/2015) and lineage III (Sheep/MelelaMlandizi/ Tanzania/ 2015) (Fig. 4.4). Sheep/MelelaMlandizi/ Tanzania/2015 showed 92% - 93% nucleotide identity with sequences from Dakawa 2013 (Tanzania), Ngorongoro 2013 (Tanzania) and Uganda 2012. This evidence linking independent outbreaks of PPR illustrates the downwards movement of the virus within Tanzania and between neighbour countries. Thus, showing the true transboundary nature of the disease. The other sequence (Goat/MelelaMlandizi/Tanzania/2015) was clustered within lineage II which was described for the first time in Tanzania by Misinzo *et al.* (2015) from an outbreak in

Tandahimba, Mtwara region (southern Tanzania). This sample showed 97% nucleotide identity with sequences from Nig/75/1, the vaccine strain. This is not the first study to report sequence identities with the vaccine strain in animals with no history of vaccination as mentioned earlier. Wang *et al*. (2014) and Misinzo *et al*. (2015) reported similar findings in studies carried out in Tanzania and China, respectively. Presence of more than one lineage in a country has also been demonstrated in countries bordering Tanzania or east African region. These include Uganda, Sudan and Ethiopia where lineages II and IV are found (Kardjadj and Luka, 2016).

In this study, no animals were introduced into the herd during the sampling periods. However, the animals were grazed in communal grazing lands with other herds. Studies have shown that contact and movement of animals from affected to unaffected areas plays an important role in transmitting the disease especially where communal grazing system is practiced (Kardjadj and Luka, 2016). *Peste des petits* ruminants virus can be transmitted when animals sneeze or cough as the virus is found in discharges from the eyes, nose and mouth of infected animals and affected animals that do not show signs of the disease (Roeder *et al.,* 1999). The severity of clinical signs, the morbidity rate, and the case fatality rate vary depending on the virulence of the virus strain, the species and breed of the host, and previous exposure of the population to the virus (Banyard *et al.*, 2010). Hence it is very important to monitor movement of animals (both illegal and legal) and use specific diagnostic tests to control further spread of PPR.

4.6 Conclusion

Antibodies to PPRV were detected and two PPRV lineages (II and III) confirmed in the farm in Melela village without animals showing typical PPR signs. This suggests that

intensive profiling of PPRV, its lineages and distribution in Tanzania is needed to understand the epidemiology and genetic diversity of the virus for control purposes.

4.7 References

Adombi, C. M., Waqas, A. G., Dundon, W., Li, S., Daojin, Y., Kakpo, L., Aplogan, G. L., Diop, M., Lo, M. M., Silber, R., Loitsch, A. and Diallo, A. (2017). *Peste des petits* ruminants in Benin: Persistence of a single virus genotype in the country for over 42 years. *Transboundary and Emerging Diseases* 64(4): 1037 - 1044.

Balamurugan, V., Rahman, H. and Munir, M. (2015). Host susceptibility to *peste des petits* ruminants virus, In: Munir, M. (Ed), *Peste des petits* ruminants virus, Springer-Verlag Berlin Heidelberg. pp 39 - 50.

Banyard, A. C., Parida, S., Batten, C., Oura, C., Kwiatek, O. and Libeau, G. (2010). Global distribution of *peste des petits* ruminants and prospects for improved diagnosis and control. *Journal of General Virology* 91(Pt 12): 2885 - 2897.

Couacy-Hymann, E., Roger, F., Hurard, C., Guillou, J. P., Libeau, G. and Diallo, A. (2002). Rapid and sensitive detection of *peste des petits* ruminants virus by a polymerase chain reaction assay. *Journal of Virological Methods* 100(1-2): 17 - 25.

Daniel, W. W. (1999). *Biostatistics: A Foundation for Analysis in the Health Sciences.* 7th edition, John Wiley and Sons, New York.

Diallo, A., Minet, C., Goff, C., Berhe, G., Albina, E., Libeau, G., Barrett, T. and le Goff, C. (2007). The threat of *peste des petits* ruminants: progress in vaccine development for disease control. *Vaccine* 25(30): 5591 - 5597.

Diop, M., Sarr, J. and Libeau, G. (2005). Evaluation of novel diagnostic tools for *peste des petits* ruminants virus in naturally infected goat herds. *Epidemiology and Infection* 133(4): 711 - 717.

Dundon, W. G., Adombi, C., Waqas, A., Otsyina, H. R., Arthur, C. T., Silber, R., Loitsch, A. and Diallo, A. (2014). Full genome sequence of a *peste des petits* ruminants virus (PPRV) from Ghana. *Virus Genes* 49(3): 497 - 501.

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95 - 98.

Kardjadj, M. and Luka, P. D. (2016). Factors affecting *peste des petits* ruminants virus (PPRV) in African countries and their counter measures. *British Journal of Virology* 3(3): 63 - 76.

Karimuribo, E. D., Loomu, P. M., Mellau, L. S. B. and Swai, E. S. (2011). Retrospective study on sero-epidemiology of *peste des petits* ruminants before its official confirmation in northern Tanzania in 2008. *Research Opinions in Animal & Veterinary Sciences* 1(3): 184 - 187.

Kgotlele, T., Macha, E. S., Kasanga, C. J., Kusiluka, L. J., Karimuribo, E. D., Van Doorsselaere, J., Wensman, J. J., Munir, M. and Misinzo, G. (2014). Partial genetic characterization of *peste des petits* ruminants virus from goats in northern and eastern Tanzania. *Transboundary and Emerging Diseases* 61(1): 56 - 62.

Kivaria, F. M., Kwiatek, O., Kapaga, A. M., Swai, E. S., Libeau, G., Moshy, W., Mbyuzi, A. O. and Gladson, J. (2013). The incursion, persistence and spread of *peste des petits* ruminants in Tanzania: Epidemiological patterns and predictions. *Onderstepoort Journal of Veterinary Research* 80(1): 593.

Kwiatek, O., Keita, D., Gil, P., Fernandez-Pinero, J., Clavero, M. A. J., Albina, E. and Libeau, G. (2010). Quantitative one-step real-time RT-PCR for the fast detection of the four genotypes of PPRV. *Journal of Virological Methods* 165(2): 168 - 177.

Kwiatek, O., Yahia, H. A., Intisar, K. S., Khalafalla, A. I., Osama, I. M., Obeida, A. A., Magdi, B. A., Osman, H. M., Taha, K. M., Zakia, A., El Harrak, M., Lhor, Y., Diallo, A., Lancelot, R., Albina, E. and Libeau, G. (2011). Asian lineage of *peste des petits* ruminants virus in Africa. *Emerging Infectious Diseases Journal* 17(7): 1223 - 1231.

Libeau, G. (2015). Current advances in serological diagnosis of *peste des petits* ruminants virus. In: Munir, M., (Ed), *Peste des petits* ruminants virus. Springer Berlin Heidelberg, pp 133 - 154.

Libeau, G., Diallo, A. and Parida, S. (2014). Evolutionary genetics underlying the spread of *peste des petits* ruminants virus. *Animal Frontiers* 4(1): 14 - 20.

Misinzo, G., Kgotlele, T., Muse, E. A., Van Doorsselaere, J., Berg, M. and Munir, M. (2015). *Peste des petits* ruminants virus lineage II and IV from goats in southern Tanzania during and outbreak in 2011. *British Journal of Virology* 2(1): 1 - 4.

Munir, M., Abubakar, M., Khan, M. T. and Abro, S. H. (2009). Comparative efficacy of single radial haemolysis test and countercurrent immune electroosmophoresis with monoclonal antibodies-based competitive ELISA for the serology of *peste des petits* ruminants in sheep and goats. *Bulgarian Journal of Veterinary Medicine* 12(4): 246 - 253.

Munir, M., Zohari, S. and Berg, M. (2012). *Molecular Biology and Pathogenesis of Peste Des Petits Ruminants Virus In: Springer Briefs In Animal Sciences.* Springer, New York. pp. 23-32.

Muse, E. A., Karimuribo, E. D., Gitao, G. C., Misinzo, G., Mellau, L. S. B., Msoffe, P. L. M., Swai, E. S. and Albano, M. O. (2012). Epidemiological investigation into the introduction and factors for spread of *peste des petits* ruminants, southern Tanzania. *Onderstepoort Journal of Veterinary Research* 79(2): 457.

Namtimba A. M. (2015). Seroprevalence and genetic characterisation of *peste des petits* ruminants virus in selected areas of Tanzania. dissertation for Award of MSc Degree at Sokoine University of Agriculture, Morogoro, Tanzania, 71pp.

Roeder, P. and Obi, T. (1999). Recognizing *peste des petits* a field manual. *Food and Agriculture organization room*, pp. 175 - 181.

Roeder, P. L., Obi, T. U., Taylor, W. and Diallo, A. (1999). Recognizing *peste des petits* ruminants: A field manual. EMPRES (Livestock) Animal Health Service FAO, Rome.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28(10): 2731 - 2739.

URT (United Republic of Tanzania). (2012). National Sample Census of Agriculture 2007/2008 Volume Ve: Regional Report; Morogoro Region. Dar es Salaam, Government Printer.

URT (United Republic of Tanzania). (2013). *Peste* Des Petits Ruminants Progressive Control And Eradication Strategy. Ministry of Livestock and Fisheries Development, Dar es Salaam.

Wang, J., Wang, M., Wang, S., Liu, Z., Shen, N., Si, W., Sun, G., Drewe, J. A. and Cai, X. (2014). *Peste des petits* ruminants virus in Heilongjiang Province, China. *Emerging Infectious Diseases* 21(4): 677-680.

Zro, K., Zakham, F., Melloul, M., Fahime, E. E. and Ennaji, M. M. (2014). A sheeppox outbreak in Morocco: isolation and identification of virus responsible for the new clinical form of disease. *BMC Veterinary Research* 10: 1 - 31.

CHAPTER FIVE

5.0 KNOWLEDGE AND RISK PRACTICES OF SMALL RUMINANTS FARMERS ON THE OCCURRENCE OF *PESTE DES PETITS* RUMINANTS IN SELECTED AREAS OF TANZANIA

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Manuscript

5.1 Abstract

Peste des petits ruminants, PPR, is one of the most economically important animal diseases of small ruminants. The disease has been earmarked as the next disease for eradication following the successful global eradication of rinderpest. One important lesson learned during the eradication of rinderpest is the involvement of livestock owners. A cross-sectional study was carried out in three regions of Tanzania to evaluate level of awareness on PPR in small ruminants' farmers and their practices in relation to this disease. A total of 107 individuals from three regions; Morogoro, Dodoma and Arusha, were interviewed using a questionnaire on demographic characteristics, knowledge and practices relating to PPR. Descriptive statistics were used, and a logistic regression model applied to evaluate the data. About 60% of the respondents had heard about PPR with most of them being from Arusha region. Small ruminants' farmers knowledge on the disease was found to be high in Arusha region and low in Morogoro region. Risk practices identified during an outbreak of PPR included selling live animals to other farmers or at the market, doing nothing and treating with available veterinary drugs. Evidently, some of the identified risk practices may be facilitating the spread of PPR because it is transmitted by direct contact or contact with contaminated fomites. One key finding was many of the respondents informed other farmers nearby about the outbreak of the disease in their herds, hence sensitizing the rest of the community. This kind of informal communication can be used to pass on messages especially relating to transmission, control and risk factors of PPR to reduce the spread of the disease. From this study, it is evident that more awareness-building among small stock farmers about PPR is needed for the successful control and ultimate eradication.

5.2 Introduction

Farmers are the primary source of surveillance information on animal diseases and health issues received by national veterinary authorities, making them important key players in the overall health of livestock. Animal health is an important aspect in livestock value chains and must be dealt with in order to maintain production levels, prevent losses and avoid transmission of animal disease (FAO, 2012). One such animal disease is *peste des petits* ruminants (PPR), a highly contagious viral disease affecting small ruminants. The disease is one of the most economically important animal diseases in areas that rely on small ruminants, especially rural communities in developing countries (EFSA, 2015).

The disease affects mainly sheep and goats and is caused by *peste des petits* ruminants virus (PPRV) (Spickler, 2015). The disease was first described in Ivory Coast in 1942, and since then it is endemic in most parts of Africa, Middle East and Asia (Parida *et al*., 2015). Tanzania and other east African countries, Kenya and Uganda, confirmed presence of PPR in the late 2000s with Tanzania confirming in 2008 (Kivaria *et al.,* 2009; Kivaria *et al*., 2013; Torsson *et al.,* 2016). This means the disease has been causing outbreaks in sheep and goats for two decades in Tanzania. Areas that have experienced severe outbreaks of the disease include Ngorongoro in the north bordering Kenya, Mtwara in the south bordering Mozambique, and Morogoro in the east of Tanzania (Swai *et al*., 2009; URT, 2013; Kgotlele *et al*., 2014; Misinzo *et al*., 2015).

The disease threatens food security and the livelihoods of small ruminants' farmers and prevents animal husbandry sectors from achieving their economic potential (Kardjadj and Luka, 2016). It is widely known that livestock is an important component in nutrition, food security, income generation and in the alleviation of hunger and poverty (Diallo *et*

al., 2007). With the global production and consumption of meat projected to continue rising, this will likely have an impact on increased risks of spread of transboundary animal diseases such as PPR as there will be increased movement of live animals and animal products for trade internationally (Sherman, 2011). In 2014, a PPR Global Control and Eradication Strategy (PPR GCES) was developed for controlling and eradicating PPR following the successful eradication of rinderpest, the Global Rinderpest Eradication Programme (GREP) (FAO, 2015). One of the lessons learned in the GREP was involving livestock owners and having strong channels of communication between livestock owners and veterinary services (FAO and OIE, 2016). Studies have shown that PPR situations and socio-economic contexts are different in each region and country, thus control and eradication programmes must reflect these differences (EFSA, 2015). Small ruminants' farmers are part of the value chain actors and their role in the spread and control of PPR is crucial as their actions or practices are more likely to influence PPR transmission and persistence in the national small ruminants' herd. In this study, interviews were conducted in selected areas of Tanzania to evaluate level of awareness of PPR by small ruminants' farmers and their practices in relation to this disease to give insight on how they contribute to the spread and control of PPR in Tanzania.

5.3 Materials and Methods

5.3.1 Study area

The study was carried out in three districts in three regions of Tanzania (Fig. 5.1). These districts were Ngorongoro district in Arusha region, in northern Tanzania bordered by Kenya to the north; Bahi district in Dodoma region, in eastern-central of Tanzania; and Mvomero district, in Morogoro region in eastern Tanzania. Arusha region is the largest region in Tanzania with a livestock density of 17.0 per km^2 and is ranked first in the total

mainland population of goats and sheep (URT, 2007a); Dodoma region is primarily semi-arid but is ranked third in livestock density at 38.7 per km^2 with livestock farming being the second major economic activity in the region (URT, 2007b); Morogoro region is ranked third in size of the total mainland area but has a very low livestock density of 4.6 per km^2 with the majority of the livestock kept by pastoral tribes that move in search of pastures (URT, 2012). The three districts were chosen purposively based on information from field extension officers and district veterinary officers based on observed clinical signs and mortality rates suspected to be PPR. The districts were chosen due to their PPR history. Morogoro and Arusha districts have a history of PPR outbreaks while Bahi has none. Questionnaires were administered in April 2016 (Mvomero district), May 2016 (Ngorongoro district) and June 2016 (Bahi district).

Figure 5.1: Map of Tanzania showing the study districts of Ngorongoro, Bahi and Mvomero in Arusha, Dodoma and Morogoro regions, respectively.

5.3.2 Study design

The study was carried out simultaneously with sero-prevalence surveys (Chapters 2, 3 and 4) to validate presence of PPR in the study areas. The study subjects were small ruminants' farmers that were present at the time of sample collection for laboratory analysis for presence of PPRV. Respondents were selected purposively after establishing if they are part of the family of the household keeping the animals and their willingness to participate in the study. A total of 107 individuals were enrolled from the three districts; that is 42 individuals from Ngorongoro district in Arusha region; 35 individuals from Mvomero district in Morogoro region and 30 individuals from Bahi district in Dodoma region.

5.3.3 Study procedure

The study was taken to establish the role small ruminants' farmers play in the introduction of PPR taking the farm as the source based on laboratory confirmation of the samples collected from animals. That is risk practices by small ruminants' farmers that may introduce PPR to the rest of the small ruminant value chain such as livestock traders, other small stock farmers and livestock transporters. A questionnaire (Appendix 2) with approximately 45 questions was developed with four sections. Section one dwelled on participant data, section two on the household and type of farming in regard to keeping animals, third section queried the general health status of animals kept and the fourth section on herd history in relation to PPR. The questionnaire was pre-tested to allow for improvements before use in the field. The questions were either open ended or dichotomous. Open ended questions were used for collection of data such as details and/or descriptions of activities or risk practices carried out, while dichotomous questions were for quick general information. The interviews were conducted by trained assistants

accompanied by the researcher. Each respondent was interviewed orally at the visit to the farm in Kiswahili, the national language of Tanzania. The data collected on the questionnaire was both qualitative and quantitative.

5.3.4 Statistical analysis

All information gathered through the questionnaire was coded by assigning a numerical value to the answers given. Questions having two possible answers were given either 0 or 1 based on response. Other questions had different levels of scores from 0, 1, 2, 3 and 4, depending on the possible outcomes question asked for. Data from the questionnaires were entered in Microsoft Excel software. Statistical analysis was conducted in Epi Info version 7 and Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistics were used for demographic characteristics, knowledge relating to PPR, attitudes and practices by farmers. Univariable logistic regression analyses were calculated and where $p < 0.2$, variables were entered in a multivariable logistic regression model until variables showed a $p \leq 0.05$. The model was investigated for interactions and confounding by adding the eliminated variables in the final model. A variable was a confounder if it changed the coefficient of the significant variables by more than 25%. The fit of the model was assessed using Hosmer-Lemeshow goodness-of-fit test.

5.4 Results

5.4.1 Demographic characteristics

The majority of the respondents were male (70%) between the ages of 21 and 45 with the average age being 42. Most of the respondents (76%) had completed formal education (primary to tertiary). All respondents interviewed used communal grazing lands. All respondents did not separate their sick animals from the rest of the herd.

5.4.2 Knowledge of *peste des petits* ruminants

Knowledge on PPR was evaluated on being able to give signs of the disease and how they differ from other diseases. Overall, knowledge on PPR was 60% that is 64 out of 107 participants. At region level, respondents who had heard about PPR were 23% (7 out of 30) in Dodoma, 100% (42 out of 42) in Arusha and 43% (15 out of 35) in Morogoro. Those who have heard about the disease, had received the information from either another farmer, a veterinarian, the media or a veterinary shop. From the small ruminants' farmers who have heard of PPR and could list a number of clinical signs, 17 respondents (5 from Morogoro and 12 from Arusha) confirmed that their animals had at one point been vaccinated against the disease, while the rest had not had their animals vaccinated against PPR. Respondents from Dodoma indicated that their animals had never been vaccinated against PPR.

5.4.3 Risk practices by small stock farmers

When respondents were asked if their animals had suffered from PPR, 37 respondents (35%) said yes while 70 (65%) said no. From the ones who said yes, 80% where from Dodoma and 20% from Arusha while there were none from Morogoro. Observations noted during an outbreak of PPR by respondents included sudden death, diarrhoea, nasal discharges, mouth lesions, labored breathing and emaciation. Practices mentioned by respondents during the outbreaks of PPR were selling the sick animals in markets or to other farmers, and treating the affected animals with available veterinary drugs. Some respondents mentioned they did nothing during the outbreak, that is did not attend to the sick animals. Asked if the district veterinary officer (DVO) was notified, 46% said yes and 54% said they did not report to the DVO. Many of the respondents however did

mention that they informed other farmers nearby about the outbreak of the disease in their herds. Multivariable logistic regression analysis showed that mixing sheep and goats in the same herd and not vaccinating animals against PPR increased the risk of animals being infected with PPR (Table 5.1). The model passed Hosmer-Lemeshow goodness-of-fit (p-value = 0.2) indicating a good fit. There were no confounders observed in the model.

Table 5.1: Results of multivariable logistic regression analysis investigating potential predictors to risk factors for PPR.

Variables	Odds Ratio		95%CI	p-value
Mixing sheep and goats in a herd	No	Ref		
	Yes	4.8	1.6 – 14.0	0.005
Mixing of different herds	No	Ref		
	Yes	0.5	0.2 – 1.6	0.08
Not vaccinating animals against PPR	No	Ref		
	Yes	8.2	2.1 – 31.8	0.002

5.5 Discussion

In this study, both women and men participated but men constituted the highest percentage (70%). Although small ruminant management systems vary among countries, rural women traditionally play a major role in this sub-sector, however, men are responsible for their disposal and are thus in charge of slaughtering and taking decisions related to their sale hence the high percentage of male respondents in this study (FAO, 2012). The average age of the respondents was 42, with 76% of them having formal education. Most animal diseases in Africa are diagnosed in the field and is by describing the clinical signs, as a way of identifying and differentiating animal diseases by farmers. Officials may also use description of clinical signs to give information about diseases to farmers. This may not be applicable to PPR as the signs are not necessarily specific to the

disease. In this study, 60% of the respondents were aware of PPR and its clinical signs. At region level, awareness of PPR by respondents was poor in Dodoma and Morogoro at 23% and 43% respectively while in Arusha all respondents were aware of the disease.

This low level of awareness southwards of the country was also noted in Tandahimba, southern Tanzania in 2011, where only 2.9% of small ruminants' farmers interviewed were aware of PPR (Malamsha, 2013) compared with those from northern Tanzania at 45.8% to 95% (Karimuribo *et al*., 2011). This shows that though the disease is moving southwards as indicated in studies done previously in Tanzania by Karimuribo *et al*. (2011), Kivaria *et al.* (2013), Misinzo *et al.* (2015) and Torsson *et al.* (2017) and the current study in chapters two (Kgotlele *et al*., 2016), three and four. The lack of knowledge/information is very critical as PPR has a high within-herd transmission rate (EFSA, 2015). To ensure successful control measures, livestock owners must be well informed and aware about the disease and its transmission to prevent further spread. This is one of the actions of the PPR eradication programme that is communication of best practices in small ruminant production and health management (FAO, 2015).

Vaccination against PPR was confirmed by a small number of respondents from Arusha and Morogoro (17 out of 77), while respondents from Dodoma have never had their animals vaccinated. This can be collaborated by official information available that PPR vaccination campaigns were carried out in Arusha and Morogoro regions in 2010 after the official confirmation (URT, 2013). This would most likely also explain the high level of awareness by small ruminants' farmers in the two regions when compared to Dodoma region.

Practices by small ruminants' farmers identified in this study during a PPR outbreak may be playing a role in the spread of PPR. Of major concern is not attending to sick animals

and selling live animals to other farmers or at the market during an outbreak. This means the virus circulates within the herd as close contact between the animals is required for effective transmission of the virus (Kumar *et al*., 2014). *Peste des petits* virus can be found in secretions from three to 22 days post infection (EFSA, 2015). This is of paramount importance as all small ruminants' farmers interviewed used communal pastures and did not separate their sick animals with the healthy ones. Trading in live animals has also been shown to be an important vehicle for transmission of infectious diseases (Domenech *et al*., 2006). The implication of animal markets has been reported by Muse *et al.* (2012), who found that lack of inadequate infrastructure especially in the local live animal markets may be facilitating transmission of PPR in Tanzania. Trade also brings about livestock theft and illegal movement of animals that plays a major role in maintaining transmission and spread of infectious diseases in many areas of East Africa (Bett *et al.,* 2009; Megersa *et al.,* 2011). The practice of purchase of live animals from markets, borrowing or hiring of animals is a big risk especially in an enzootic area for the spread of a disease such as PPR (Kardjadj and Luka, 2016). Another practice mentioned by small ruminants' farmers was use of veterinary drugs to reduce the intensity of the disease in the herd. This reduced the number of deaths in a flock as treatment for bacterial and parasitic complications decreases mortality in affected herds (Aiello and Moses, 2011). However, this practice does not necessarily mean the herd will not transmit the disease if they come into contact with a naïve herd or animals, thus continue spreading the virus.

Mixing of sheep and goats was found to increase the risk of animals getting the virus. All small ruminants' farmers interviewed mixed sheep and goats in their herds. Many authors believe that although PPRV infects sheep and goats, the severity of the clinical

signs is more predominant in goats than sheep (Balamurugan *et al*., 2015). This high incidence in goats than sheep was also noted by small ruminants' farmers whose animals had suffered from PPR. There is an inconsistency in research findings where some found more pronounced clinical signs in sheep than goats (Singh *et al*., 2004). Thus, the best practice would be to advice farmers to always separate the sick from the healthy animals especially where both species are kept in a herd.

The other risk practice identified in this study was not vaccinating animals against PPR. From this study, vaccination coverage is low as only small ruminants' farmers in Arusha and Morogoro had their animals vaccinated against PPR. Sheep and goats vaccinated against PPR develop an active immunity against the disease for at least three years (OIE, 2013). In countries where the disease is endemic in nature, the most commonly employed control method is by increasing the immunity level through extensive vaccination campaigns (EFSA, 2015). As evidenced during the GREP, one of the key activities was the use of a highly efficacious vaccine (FAO and OIE, 2016). Evidence from countries carrying out PPR vaccinations show that mass vaccinations of animals should be carried out a month before the expected seasonal movements due to factors such as dry spells, increased market activities or major religious festivals (Kumar *et al*., 2014).

Another finding of importance in this study was that many of the respondents whose animals had suffered from PPR informed other farmers nearby about the outbreak of the disease in their herds. This informal communication may be exploited by veterinary services to help control the disease as it was noted with the eradication of rinderpest (FAO and OIE, 2016). Establishing and/or strengthening passive surveillance would help

in early detection of the appearance of the disease or virus incursion as it is known that passive surveillance is the most likely way in which an introduction of the disease might be detected (EFSA, 2015). Of concern from the findings was lack of full disclosure of the outbreak to DVOs. Only 20% of livestock producers are able to access extension services in Tanzania, hence the low reporting of an outbreak to a DVO (URT, 2006). This is where mobile technology would greatly bridge the gap between livestock farmers and veterinary officials for delivery of comprehensive extension services (URT, 2006).

5.6 Conclusion

Overall, small ruminants' farmers who experienced an outbreak were aware of PPR and its clinical signs. The small ruminants' farmers were aware and knowledgeable about the disease, but risky practices were carried out during outbreaks. This shows that there is still a knowledge gap on best practices during disease outbreaks. More awareness building among small ruminants' farmers about PPR is needed as it is crucial for the successful control and ultimately eradication of the disease. The awareness would further establish communication between the livestock owners and various stakeholders and build mutual respect that is essential for future collaborations and endeavors of control of other diseases of small ruminants.

5.7 References

Aiello, S. E. and Moses, M. A. (2011). The Merck Veterinary Manual online. [http://www.merckmanuals.com/vet/search.html?qt=peste+des+petits+ruminants&start=1&context=%2Fvet] site visited on 5/3/12.

Balamurugan, V., Rahman, H. and Munir, M. (2015). Host susceptibility to *peste des petits* ruminants virus, In: Munir, M. (Ed), *Peste Des Petits Ruminants Virus*, Springer-Verlag Berlin Heidelberg. pp 39 - 50.

Bett, B., Jost, C., Allport, R. and Mariner, J. (2009). Using participatory epidemiological techniques to estimate the relative incidence and impact on livelihoods of livestock diseases amongst nomadic pastoralists in Turkana South District, Kenya. *Preventive Veterinary Medicine* 90(3-4): 194 - 203.

Diallo, A., Minet, C., le Graff, C., Berhe, G., Albina, E., Libeau, G. and Barrett, T. (2007). The threat of *peste des petits* ruminants progress in vaccine development for disease control. *Vaccine* 25(30): 5591 - 5597.

Domenech, J., Lubroth, J., Eddi, C., Martin, V. and Roger, F. (2006). Regional and international approaches on prevention and control of animal transboundary and emerging diseases. *Annals of the New York Academy of Sciences* 1081: 90 - 107.

EFSA (EFSA panel on Animal Health and Welfare). (2015). Scientific opinion on *peste des petits* ruminants. *European Food Safety Authority Journal* 13(1): 94.

FAO (2012). Designing and implementing livestock value chain studies – A practical aid for Highly Pathogenic and Emerging Disease (HPED) control. FAO Animal Production and Health Guidelines No. 10. Rome.

FAO (2015). poster. [www.fao.org/ppr, BC274e/1/12.15] site visited on 1/12/2015.

FAO and OIE (2016). *Peste des petits* ruminants global eradication programme; Contributing to food security, poverty alleviation and resilience - Five years (2017–2021). Food and Agriculture Organization, Rome.

Kardjadj, M. and Luka, P. D. (2016). Factors affecting PPRV in African countries and their countermeasures. *British Journal of Virology* 3(3): 63 - 76.

Karimuribo, E. D., Loomu, P. M., Mellau, L. S. B. and Swai, E. S. (2011). Retrospective study on sero-epidemiology of *peste des petits* ruminants before its official confirmation in northern Tanzania in 2008. *Research Opinions in Animal and Veterinary Sciences* 1(3): 184 - 187.

Kgotlele, T., Kasanga, C. J., Kusiluka, L. J. M. and Misinzo, G. (2014). Preliminary investigation on presence of *peste des petits* ruminants in Dakawa, Mvomero district, Morogoro region, Tanzania. *Onderstepoort Journal of Veterinary Research* 81(2): 1 - 3.

Kgotlele, T., Torsson, E., Kasanga, C. J., Wensman, J. J., and Misinzo, G. (2016). Seroprevalence of *peste des petits* ruminants virus from samples collected in different regions of Tanzania in 2013 and 2015. *Journal of Veterinary Science and Technology* 7: 394.

Kivaria, F. M., Kwiatek, O., Kapaga, A. M., Geneviève, L., Mpelumbe-Ngeleja, C. A. R. and Tinuga, D. K. (2009). Serological and virological investigations on an emerging peste des petits ruminants virus infection in goats and sheep in Tanzania. In: *Proceedings of the 27th Tanzania Veterinary Association Scientific Conference*. 04 - 06 December 2009, Arusha, Tanzania.

Kivaria, F. M., Kwiatek, O., Kapaga, A. M., Swai, E. S., Libeau, G., Moshy, W., Mbyuzi, A. O. and Gladson, J. (2013). The incursion, persistence and spread of *peste des petits* ruminants in Tanzania: Epidemiological patterns and predictions. *Onderstepoort Journal of Veterinary Research* 80(1): 593.

Kumar, N., Maherchandani, S., Kashyap, S. K., Singh, S. V., Sharma, S., Chaubey, K. K. and Ly, H. (2014). *Peste des petits* ruminants virus infection of small ruminants: A comprehensive review. *Viruses* 6(6): 2287 - 2327.

Malamsha, N. M. (2013). *Peste-des-petits*-ruminants status in Tanzania. In: Workshop on PPR Prevention and Control in the SADC Region, 10 - 12 June, 2013, Dar es Salaam, Tanzania.

Megersa, B., Biffa, D., Belina, T., Debela, E., Regassa, A., Abunna, F., Rufael, T., StubsjÃ, S. M. and Skjerve, E. (2011). Serological investigation of *peste des petits* ruminants (PPR) in small ruminants managed under pastoral and agro-pastoral systems in Ethiopia. *Small Ruminant Research* 97(1-3): 134 - 138.

Misinzo, G., Kgotlele, T., Muse, E. A., van Doorsselaere, J., Berg, M. and Munir, M. (2015). *Peste des petits* ruminants virus lineage II and IV from goats in southern Tanzania during an outbreak in 2011. *British Journal of Virology* 2(1): 1 - 4.

Muse, E. A., Karimuribo, E. D., Gitao, G. C., Misinzo, G., Mellau, L. S. B., Msoffe, P. L. M., Swai, E. S. and Albano, M. O. (2012). Epidemiological investigation into the introduction and factors for spread of *peste des petits* ruminants, southern Tanzania. *Onderstepoort Journal of Veterinary Research* 79(2): 457.

OIE (2013). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. [http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.11_PPR.pd] site visited on 22/5/2012.

Parida, S., Muniraju, M., Mahapatra, M., Muthuchelvan, D., Buczkowski, H. and Banyard, A. C. (2015). *Peste des petits* ruminants. *Veterinary Microbiology* 181(1-2): 90 - 106.

Sherman, D. M. (2011). The spread of pathogens through trade in small ruminants and their products. *Revue scientifique et technique* (International Office of Epizootics) 30(1): 207 - 217.

Singh, R. P., Saravanan, P., Sreenivasa, B. P., Singh, R. K. and Bandyopadhyay, S. K. (2004). Prevalence and distribution of *peste des petits* ruminants virus infection in small ruminants in India. *Revue scientifique et technique* (International Office of Epizootics) 23(3): 807 - 819.

Spickler, A. R. (2015). *Peste des petits* ruminants http://www.cfsph. iastate.edu/ DiseaseInfo/factsheets.php site visited on 15/02/2018.

Swai, E. S., Kapaga, A., Kivaria, F., Tinuga, D., Joshua, G. and Sanka, P. (2009). Prevalence and distribution of *peste des petits* ruminants virus antibodies in various districts of Tanzania. *Veterinary Research Communications* 33(8): 927 - 936.

Torsson, E., Kgotlele, T., Berg, M., Mtui-Malamsha, N., Swai, E. S., Wensman, J. J. and Misinzo, G. (2016). History and current status of *peste des petits* ruminants virus in Tanzania. *Infection Ecology and Epidemiology* 6: 32701.

Torsson, E., Berg, M., Misinzo, G., Herbe, I., Kgotlele, T., Päärnib, M., Roosa, N., Blomströma, A., Ståhld, K. and Wensman, J. J. **(**2017). Seroprevalence and risk factors for *peste des petits* ruminants and selected differential diagnosis in sheep and goats in Tanzania. *Infection Ecology and Epidemiology* 7: 1368336.

URT (2006). National livestock policy, Ministry of Livestock Development, Dar es Salaam, Government Printer.

URT (2007a). *National Sample Census of Agriculture 2002/2003 Volume Va: Regional Report: Arusha Region*. Government Printer, Dar es Salaam.

URT (2007b). *National Sample Census of Agriculture 2002/2003 Volume Va: Regional Report: Dodoma Region*. Government Printer, Dar es Salaam.

URT (2012). *National Sample Census of Agriculture 2007/2008 Volume Ve: Regional Report; Morogoro Region.* Government Printer, Dar es Salaam.

URT (2013). *Peste Des Petits Ruminants Progressive Control and Eradication Strategy*. Ministry of Livestock and Fisheries Development, Dar es Salaam,

CHAPTER SIX

6.0 GENERAL DISCUSSION

From this study it shows that *peste des petits* ruminants (PPR) is widely prevalent in small ruminants in different regions of Tanzania. All study regions had seropositive cases. The overall observed true seroprevalence of 27.1% ($CI_{95\%}$: 25.6 - 28.5) in 2013 and 2015 is low compared to previous reports in 2009 at 45.4% (Swai *et al.,* 2009) and 2012 at 31.0% (Muse *et al*., 2012), respectively. Though the overall true seroprevalence is low, the figure is highly significant in a country with an estimated population of sheep and goats over 20 million (URT, 2015). True seroprevalence by regions varies with some regions registering true seroprevalence of less than 20%. This difference may be attributed to vaccination campaigns carried out in 2008 (Arusha region) and 2010 (Morogoro and Mtwara regions) (URT, 2013). Small ruminants vaccinated on a large scale with PPR vaccines will still test positive for antibodies to *peste des petits* ruminants virus (PPRV) because the cELISA test cannot discriminate from previously PPRV infected animals and vaccinated animals (Libeau, 2015). This is because vaccinated animals are known to have lifelong immunity after vaccinations. The use of antibiotics in managing clinical cases is also believed to increase survival rate of sick animals (Karimuribo *et al*., 2011) thus the surviving animals will carry antibodies to PPRV. These two factors may have contributed to the differences in seroprevalences.

The seropositivity difference between sheep and goats remains unclear in literature. In this study there were no statistical differences of seropositivity between species. This is in

contradiction with some studies, including one carried out in Tanzania (Swai *et al*., 2009), which reported a higher seroprevalence in goats than in sheep and linked it to higher fecundity in goats compared to sheep (Abdalla *et al*., 2012). Other studies have reported higher seroprevalence in sheep than goats, attributing it to lower number of sheep sampled or due to the fact that goats are often affected more severely by the disease hence die prior to sampling (Megersa *et al.*, 2011). There was also no statistical difference between males and females. This finding is different from another study done in Tanzania where females were more likely to be seropositive than males (Torsson *et al*., 2017). It is believed that females are used in reproduction hence kept longer in the herd than males, therefore have a longer risk period for exposure in the herd. Stress associated with pregnancy and milk production could also predispose them to infection (Aziz-Ul *et al*., 2016). Therefore, more investigations are needed to further determine the variation between the species and sexes. There was statistical difference between animals under 2 years less likely to be seropositive than those above 2 years. Studies have shown that differences noted between the age groups are most likely due to younger animals loosing their aquired passive immunity after three months and the older ones having life long immunity after survival of infection (Kul *et al*., 2015).

Observed clinical signs in the examined animals were mostly nasal discharges and diarrhoea in both sheep and goats. In one study area, only goats exhibited excess nasal discharges but did not show any other clinical presentations typical of PPR. This can be attributed to the type of breed found in the district which is mainly indigenous. Clinical signs can be affected by type of breed of sheep and goats as some breeds fail to develop characteristic clinical signs especially indigenous goats and sheep (Munir *et al*., 2012). Failure of some breeds to exhibit clinical signs can lead to spread of the virus as animals

would be mistaken to be apparently healthy hence can either be sold or transported, spreading the virus further.

The clinical signs observed in this study are expressed in many infectious diseases of small ruminants. Diseases of small ruminants that present similar observed clinical signs include; PPR, pneumonic pasteurellosis and contagious caprine pleuropneumonia (CCPP) for nasal discharges; PPR, coccidiosis or gastro-intestinal helminth infestations for diarrhea; and contagious ecthyma, sheep pox and goat pox for nodular lesions (Zro *et al*., 2014). In Tanzania, difficulty in differentiating clinical signs in sheep and goats has been mentioned as a major limiting factor in diagnosis especially for PPR and CCPP (Mbyuzi *et al*., 2014). In such cases where specific manifestations of the diseases are absent, reliable laboratory tests are needed.

Four pathogens were investigated in the study and confirmed in the laboratory. These were PPR virus (PPRV), *Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp), *Pasteurella multocida (P. multocida)* and *Capripoxvirus* (CaPV). All four pathogens were detected from nasal swabs, including co-infections of PPRV and Mccp, PPRV and CaPV, PPRV and *P. multocida*, and Mccp and CaPV. Co-infection of PPRV with other viral and bacterial infections in small ruminants have been demonstrated before in other studies in different parts of the world (Kul *et al*., 2015). The co-infections detected in this study may have provided complications during field diagnosis that resulted in the persistence and severity in the affected flock. Thus, laboratory confirmation is critical for appropriate interventions to be made for the different diseases of sheep and goats especially regarding PPR.

Using conventional polymerase chain reaction (PCR) for detection of the nucleoprotein, PPRV was isolated from collected blood and swabs. Two samples were sent for sequencing to determine lineages. The two sequenced samples were clustered within lineage II (Goat/MelelaMlandizi/Tanzania/2015) and lineage III (Sheep/MelelaMlandizi/ Tanzania/2015). Sheep/MelelaMlandizi/ Tanzania/2015 showed 92% - 93% nucleotide identity with sequences from Dakawa 2013 (Tanzania), Ngorongoro 2013 (Tanzania) and Uganda 2012. This evidence linking independent outbreaks of PPR illustrates the downwards movement of the virus within Tanzania and between neighbour countries. Thus, showing the true transboundary nature of the disease. The other sequence (Goat/MelelaMlandizi/Tanzania/2015) was clustered within lineage II which was described for the first time in Tanzania by Misinzo *et al.* (2015) from an outbreak in Tandahimba, Mtwara region (southern Tanzania). This sample showed 97% nucleotide identity with sequences from Nig/75/1, the vaccine strain. This is not the first study to report sequence identities with the vaccine strain in animals with no history of vaccination as mentioned earlier. Wang *et al*. (2014) and Misinzo *et al*. (2015) reported similar findings in studies carried out in Tanzania and China, respectively. Presence of more than one lineage in a country has also been demonstrated in countries bordering Tanzania. These include Uganda, Sudan and Ethiopia where lineages II and IV are found (Kardjadj and Luka, 2016).

The study looked into practices by small ruminants farmers that my influencing spread and control of PPR. Farmers are part of the value chain actors and their role is crucial as their actions or practices are more likely to influence PPR transmission and persistence in the national small ruminants.

Both women and men participated in the study but men constituted the highest percentage at 70%. Although small ruminant management systems vary among countries, rural women traditionally play a major role in this sub-sector with men responsible for slaughtering and taking decisions related to their sale (FAO, 2015) hence the high percentage of male respondents in this study. The average age of the respondents was 42, with 76% of them having formal education.

Sixty percent of respondents were aware of PPR and its clinical signs. At region level, awareness of PPR by respondents was poor in Dodoma and Morogoro at 23% and 43% respectively, while in Arusha all respondents were aware of the disease. This low level of awareness southwards of the country was also noted in Tandahimba, southern Tanzania in 2011, where only 2.9% of small ruminants' farmers interviewed were aware of PPR (Malamsha, 2013) compared with those from northern Tanzania at 45.8% to 95% (Karimuribo *et al*., 2011). This shows that though the disease is moving southwards as indicated in studies done previously in Tanzania by Karimuribo *et al*. (2011), Kivaria *et al.* (2013), Misinzo *et al.* (2015) and Torsson *et al.* (2017). The lack of knowledge/information is very critical as PPR has a high within-herd transmission rate (EFSA, 2015). To ensure successful control measures, livestock owners must be well informed and aware about the disease and its transmission to prevent further spread.

Practices by small ruminants' farmers identified in this study during a PPR outbreak included not attending to sick animals and selling live animals to other farmers or at the market. This means the virus circulates within the herd as close contact between the animals is required for effective transmission of the virus (Kumar *et al*., 2014). *Peste des petits* virus can be found in secretions from three to 22 days post infection (EFSA, 2015). This is of paramount importance as all small ruminants' farmers interviewed used

communal pastures and did not separate their sick animals with the healthy ones. Trading in live animals has also been shown to be an important vehicle for transmission of infectious diseases (Domenech *et al*., 2006). The implication of animal markets has been reported by Muse *et al.* (2012), who found that lack of inadequate infrastructure especially in the local live animal markets may be facilitating transmission of PPR in Tanzania. Trade also brings about livestock theft and illegal movement of animals that plays a major role in maintaining transmission and spread of infectious diseases in many areas of East Africa (Megersa *et al.*, 2011). The practice of purchase of live animals from markets, borrowing or hiring of animals is a big risk especially in an enzootic area for the spread of a disease such as PPR (Kardjadj and Luka, 2016). Another practice mentioned by small ruminants' farmers was use of veterinary drugs to reduce the intensity of the disease in the herd. This reduced the number of deaths in a flock as treatment for bacterial and parasitic complications decreases mortality in affected herds (Aiello and Moses, 2011). However, this practice does not necessarily mean the herd will not transmit the disease if they come into contact with a naïve herd or animals, thus continue spreading the virus.

Mixing of sheep and goats was found to increase the risk of animals getting the virus. All small ruminants' farmers interviewed mixed sheep and goats in their herds. Many authors believe that although PPRV infects sheep and goats, the severity of the clinical signs is more predominant in goats than sheep (Balamurugan *et al*., 2015). This high incidence in goats than sheep was also noted by small ruminants' farmers whose animals had suffered from PPR. There is an inconsistency in research findings where some found more pronounced clinical signs in sheep than goats (Singh *et al*., 2004). Thus, the best practice

would be to advice farmers to always separate the sick from the healthy animals especially where both species are kept in a herd.

The other risk practice identified in this study was not vaccinating animals against PPR. From this study, vaccination coverage is low as only a small number of small ruminants' farmers in Arusha and Morogoro had their animals vaccinated against PPR. In countries where the disease is endemic in nature, the most commonly employed control method is by increasing the immunity level through extensive vaccination campaigns (EFSA, 2015). As evidenced during the GREP, one of the key activities was the use of a highly efficacious vaccine (FAO and OIE, 2016). Another finding of importance in this study was that many of the respondents whose animals had suffered from PPR informed other farmers nearby about the outbreak of the disease in their herds. This informal communication may be exploited by veterinary services to help control the disease as it was noted with the eradication of rinderpest (FAO and OIE, 2016).

Peste des petits ruminants virus can be transmitted when animals sneeze or cough as the virus is found in discharges from the eyes, nose and mouth of infected animals and affected animals that do not show signs of the disease (Roeder *et al.,* 1999). The severity of clinical signs, the morbidity rate, and the case fatality rate vary depending on the virulence of the virus strain, the species and breed of the host, and previous exposure of the population to the virus (Banyard *et al.*, 2010). Hence it is very important to monitor movement of animals (both illegal and legal) and use specific diagnostic tests to control further spread of PPR.

References

Abdalla, A. S., Majok, A. A., El Malik, K. H. and Ali, A. S. (2012). Sero-prevalence of *peste des petits* ruminants virus (PPRV) in small ruminants in Blue Nile, Gadaref and North Kordofan States of Sudan. *Journal of Public Health and Epidemiology* 4(3): 59 - 64.

Aiello, S. E. and Moses, M. A. (2011). The Merck Veterinary Manual online. [http://www.merckmanuals.com/vet/search.html?qt=peste+des+petits+ruminants&start=1&context=%2Fvet] site visited on 5/3/12.

Aziz-Ul-Rahman, Abubakar, M., Rasool, M. H., Manzoor, S., Saqalein, M., Rizwan, M., Munir, M., Ali, Q. and Wensman, J. J. (2016). Evaluation of risk factors for *peste des petits* ruminants virus in sheep and goats at the wildlife-livestock interface in Punjab Province, Pakistan. *BioMed Research International* 2016(2016): 7826245.

Balamurugan, V., Rahman, H. and Munir, M. (2015). Host susceptibility to *peste des petits* ruminants virus, In: Munir, M. (Ed), *Peste des petits* ruminants virus, Springer-Verlag Berlin Heidelberg. pp 39 - 50.

Banyard, A. C., Parida, S., Batten, C., Oura, C., Kwiatek, O. and Libeau, G. (2010). Global distribution of *peste des petits* ruminants and prospects for improved diagnosis and control. *Journal of General Virology* 91(Pt 12): 2885 - 2897.

Domenech, J., Lubroth, J., Eddi, C., Martin, V. and Roger, F. (2006). Regional and international approaches on prevention and control of animal transboundary and emerging diseases. *Annals of the New York Academy of Sciences* 1081: 90 - 107.

EFSA (2015). Scientific opinion on *peste des petits* ruminants. *European Food Safety Authority Journal* 13(1): 94.

FAO (2015). Poster. [www.fao.org/ppr, BC274e/1/12.15] site visited on 1/12/2015.

FAO and OIE (2016). *Peste Des Petits Ruminants Global Eradication Programme; Contributing to Food Security, Poverty Alleviation and Resilience - Five Years (2017–2021).* Food and Agriculture Organization of the United Nations and the World Organisation for Animal Health, Rome. 80pp.

Kardjadj, M. and Luka, P. D. (2016). Factors affecting PPRV in African countries and their countermeasures. *British Journal of Virology* 3(3): 63 - 76.

Karimuribo, E. D., Loomu, P. M., Mellau, L. S. B. and Swai, E. S. (2011). Retrospective study on sero-epidemiology of *peste des petits* ruminants before its official confirmation in northern Tanzania in 2008. *Research Opinions in Animal and Veterinary Sciences* 1(3): 184 - 187.

Kivaria, F. M., Kwiatek, O., Kapaga, A. M., Swai, E. S., Libeau, G., Moshy, W., Mbyuzi, A. O. and Gladson, J. (2013). The incursion, persistence and spread of *peste des petits* ruminants in Tanzania: Epidemiological patterns and predictions. *Onderstepoort Journal of Veterinary Research* 80(1): 593.

Kul, O., Atmaca, H. T. and Munir, M. (2015). Pathology of *peste des petits* ruminants virus infection in small ruminants and concurrent infections. In: Munir, M. (Ed) *Peste des petits* ruminants virus. Springer-Verlag Berlin Heidelberg, pp 119 - 131.

Kumar, N., Maherchandani, S., Kashyap, S. K., Singh, S. V., Sharma, S., Chaubey, K. K. and Ly, H. (2014). *Peste des petits* ruminants virus infection of small ruminants: A comprehensive review. *Viruses* 6(6): 2287 - 2327.

Libeau, G. (2015). Current advances in serological diagnosis of *peste des petits* ruminants virus. In: Munir, M., (Ed), *Peste* Des Petits Ruminants Virus. Springer Berlin Heidelberg. Pp. 133 - 154.

Malamsha, N. M. (2013). *Peste-des-petits*-ruminants (PPR) status in Tanzania. In: Workshop on PPR Prevention and Control in the SADC Region, 10 - 12 June, 2013, Dar es Salaam, Tanzania.

Mbyuzi, A. O., Komba, E. V., Kimera, S. I. and Kambarage, D. M. (2014). Sero-prevalence and associated risk factors of *peste des petits* ruminants and contagious caprine pleuro-pneumonia in goats and sheep in the Southern Zone of Tanzania. *Preventive Veterinary Medicine* 116(2): 138 - 144.

Megersa, B., Biffa, D., Belina, T., Debela, E., Regassa, A., Abunna, F., Rufael, T., StubsjÃ, S. M. and Skjerve, E. (2011). Serological investigation of *peste des petits* ruminants (PPR) in small ruminants managed under pastoral and agro-pastoral systems in Ethiopia. *Small Ruminant Research* 97(3): 134 - 138.

Misinzo, G., Kgotlele, T., Muse, E. A., van Doorsselaere, J., Berg, M. and Munir, M. (2015). *Peste des petits* ruminants virus lineage II and IV from goats in southern Tanzania during an outbreak in 2011. *British Journal of Virology* 2(1): 1 - 4.

Munir, M., Zohari, S. and Berg, M. (2012). *Molecular Biology and Pathogenesis of Peste Des Petits Ruminants Virus In: Springer Briefs In Animal Sciences.* Springer, New York. pp. 23-32.

Muse, E. A., Karimuribo, E. D., Gitao, G. C., Misinzo, G., Mellau, L. S. B., Msoffe, P. L. M., Swai, E. S. and Albano, M. O. (2012). Epidemiological investigation into the introduction and factors for spread of *peste des petits* ruminants, southern Tanzania. *Onderstepoort Journal of Veterinary Research* 79(2): 457.

Roeder, P. L., Obi, T. U., Taylor, W. and Diallo, A. (1999). Recognizing *peste des petits* ruminants: A field manual. EMPRES (Livestock) Animal Health Service FAO, Rome.

Singh, R. P., Saravanan, P., Sreenivasa, B. P., Singh, R. K. and Bandyopadhyay, S. K. (2004). Prevalence and distribution of *peste des petits* ruminants virus infection in small ruminants in India. *Revue Scientifique ET Technique* 23(3): 807 - 819.

Swai, E. S., Kapaga, A., Kivaria, F., Tinuga, D., Joshua, G. and Sanka, P. (2009). Prevalence and distribution of *peste des petits* ruminants virus antibodies in various districts of Tanzania. *Veterinary Research Communications* 33(8): 927 - 936.

Torsson, E., Berg, M., Misinzo, G., Herbe, I., Kgotlele, T., Päärnib, M., Roosa, N., Blomströma, A., Ståhld, K. and Wensman, J. J. **(**2017). Seroprevalence and risk factors for *peste des petits* ruminants and selected differential diagnosis in sheep and goats in Tanzania. *Infection Ecology and Epidemiology* 7: 8pp.

URT (2013). *Peste Des Petits Ruminants Progressive Control And Eradication Strategy*. Ministry of Livestock and Fisheries Development, Dar es Salaam. 80pp.

Wang, J., Wang, M., Wang, S., Liu, Z., Shen, N., Si, W., Sun, G., Drewe, J. A. and Cai, X. (2014). *Peste des petits* ruminants virus in Heilongjiang Province, China. *Emerging Infectious Diseases* 21(4): 677-680.

Zro, K., Zakham, F., Melloul, M., Fahime, E. E. and Ennaji, M. M. (2014). A sheeppox outbreak in Morocco: isolation and identification of virus responsible for the new clinical form of disease. *BMC Veterinary Research* 10: 31.

CHAPTER SEVEN

7.0 CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

From the results of this thesis, it can be concluded that:

Seroprevalences evidenced in the study regions show that PPRV is circulating and spreading to other geographical areas that had not reported its presence before. Serological studies also showed that age (> 2 years) was a risk factor identified for PPRV seropositivity while species and sex were not identified as risk factors.

Infection of PPRV and co-infection with other pathogens causing similar clinical signs in sheep and goats in Ngorongoro district highlights the unreliability of using clinical signs to diagnose PPR in the field without laboratory confirmation.

The two lineages, II and III confirmed circulating in a farm in Melela Mlandizi village, Morogoro region in the same herd demonstrates the genetic diversity of PPRV in sheep and goats in an endemic setting in Tanzania.

Livestock keeping communities were aware of PPR and its clinical signs in Arusha, Dodoma and Morogoro regions but risky practices such as trading live animals may be facilitating the spread of the disease in Tanzania.

7.2 Recommendations

It is recommended that:

i. Control measures such as vaccination should be intensified with a wide coverage, taking into consideration the geographical distribution of the disease in Tanzania. Consideration of annual vaccinations to improve the herd immunity to levels that can contain the spread of PPR with emphasis of vaccinating young sheep and goats (≤ 2 years) as increase in age was noted to be a risk factor to being PPRV seropositive.

ii. Facilitate research into PPR and other pathogens that cause diseases in small ruminants found in Tanzania to improve their control by linking them to PPR control measures and eradication programme. This would also result in the possibility of developing dual vaccines that could be used to protect sheep and goats against both PPR and other diseases of small ruminants common in Tanzania.

iii. Full genome sequencing of lineages II and III virus strains from Tanzania to study their genetic diversity and how they compare to other strains of the same lineages.

iv. Awareness raising campaigns for farmers on recognizing PPR and best practices during an outbreak should be made a priority. This would mean farmers can be instrumental in early detection, a necessary condition for rapid response and effective management of outbreaks of PPR with the ultimate goal of eradicating PPR in Tanzania, regionally and globally.

APPENDICES

Appendix 1: Research Methodologies

Chapter 2 research methodology

Study design and sample collection

A total of 3838 serum samples from 118 villages collected from sheep and goats in 14 regions of Tanzania in 2013 and 2015 were used. For 2013, samples were randomly selected from frozen sera (kept at -20°C). Serum samples collected in 2013 were collected by the Ministry of Livestock and Fisheries (MLF) from different regions to determine presence of PPR before a vaccination campaign and sample collection employed a multistage sampling strategy as described by Kivaria *et al*. (2013). Briefly, the first level of selection was the regions where regions were purposely selected because of their presence of farming activities characterized by extensive livestock movement. Within each of the selected regions, specific districts were also purposely selected. From the selected districts, villages were randomly selected. Within each of the selected villages, about 40 animals were randomly selected. The sample size was computed as:

$$n = z^2_{\alpha 0.05} \times [p \times (1\text{-}p)/L^2]$$

where:

z = 1.96

p = 0.32 (prior prevalence)

L = 0.05 (the desired level of accuracy)

The required sample size was calculated as n = 334.37.

As PPR is a highly contagious disease, to obtain similar accuracy to that with simple random sampling the sample size was recalculated as:

$$n_{new} = n \times [1 + \rho \times (m - 1)]$$

where

n = 334.37

ρ = 0.156 (estimated average rate of PPR homogeneity)

m = 40 (average number of animals to be sampled from each village).

The new sample size was equal to 2,360.

For this study, samples retrieved from the freezer from 2013 were 3392 (2617 goats and 775 sheep) from the 11 regions. The other samples (n = 446) used in the study were collected in 2015 from 3 regions comprising of 269 goats and 177 sheep. All samples used in this study were collected from apparently healthy sheep and goats, that is animals did not show any clinical signs associated with PPR. Sex and age of sampled animals could not be retrieved from information stated in the submission forms.

Detection of *peste des petits* ruminants antibodies using competitive enzyme-linked immunosorbent assay (cELISA)

Sera were tested for antibodies against PPRV using a competitive ELISA kit (cELISA) (IDVet, Grabels, France) that detects anti-PPRV nucleoprotein antibodies. The test was performed according to manufacturer's instructions. Samples presenting a competition percentage of less than or equal to 50% were considered positive, greater than 60% were considered negative while greater than 50% or equal to 60% were considered doubtful for PPRV antibodies. In the statistical analysis a doubtful result was considered as negative.

Statistical analysis

True prevalence was calculated based on apparent prevalence, sensitivity and specificity of the diagnostic test used as described by Rogan and Gladen (1978). The kit used has sensitivity of 94.5% and specificity of 99.4% (Libeau *et al*., 1995) and were used as follows:

$$\text{true prevalence rate} = \frac{\text{apparent prevalence rate + kit specificity -1}}{\text{kit sensitivity + kit specificity - 1}}$$

The odds ratio was calculated to assess the association between being seropositive or seronegative for PPR and animal species (Altman, 1991) with the value of $p < 0.05$ considered significant. Other factors such as sex and age could not be calculated as the information was missing in the submission forms of samples.

Chapter 3 research methodology

Study area

The aim of this study was to investigate PPR and some of its differential diagnoses to determine causes of a respiratory disease outbreak in sheep and goats in Loliondo area in Ngorongoro district of Arusha region in Tanzania and help officials take correction measures based on confirmed causes of the outbreak. The study was conducted in Ngorongoro district, one of the seven districts of Arusha region in northern Tanzania. The villages involved in the investigation were Sukenyan, Mondorosi, Ololosokwan and Enguserosambu. The villages are inhabited by Maasai and Sonjo ethnic groups that are traditionally pastoralists and agro-pastoralists, respectively. The study area was chosen after a reported outbreak of a disease affecting sheep and goats with high mortality rates in May 2016. The animals were said to present respiratory distress, diarrhea and

mucopurulent nasal discharges. The outbreak was reported to have been going on for four months before collection of samples for this study.

Study animals and samples

Sheep and goats were randomly examined for clinical signs from the four villages. Animals which were clinical sick were purposively sampled for this study. Using prevalence of 80% as given by the District Veterinary Officer (DVO), the sample size was determined using the formula prescribed by Israel (2013):

$$n_0 = \frac{Z^2 pq}{e^2}$$

where:

n_o = desired sample size

Z = Z statistic confidence level of 95% (1.96)

p = prevalence of the attribute present in the population (80%)

q = probability of having no attribute in the population (1-p)

e = marginal error (5%)

This gave the sample size of 245 samples. A total of 301 samples were collected. Samples collected were 240 sera (from 59 sheep and 181 goats) and 61 nasal swabs (from 38 sheep and 23 goats). Age and sex of sampled animals were recorded during sample collection.

Serological assay

Serum samples were tested for presence of antibodies to PPRV using commercial kit from ID screen® PPR competition ELISA (IDVet, Grabels, France). The kit was used and interpreted according to manufacturer's instructions. Briefly, 25 µL of samples and controls were added to the antigen coated wells and incubated for 45 mins at 37°C. The plates were washed three times with washing buffer. Then, 100 µL of anti-PPRV

nucleoprotein horseradish peroxidase conjugate was applied to the wells and again incubated for 30 mins to cover unbound antigens. Washing was done again three times followed by adding 100 µL of substrate solution and incubated for 15 mins. The reaction was stopped with 100 µL stop solution and reading was taken at 450nm on ELISA reader (Multiscan Ex, Thermo Corp, USA). Samples presenting a competition percentage of less than or equal to 50% were considered positive, greater than 60% were considered negative while greater than 50% or equal to 60% were considered doubtful for PPRV antibodies. In the statistical analysis a doubtful result was considered as negative.

Serological study was chosen for this study to estimate widespread infection of PPR before determining presence of the virus in the samples. Serological studies have shown that animals can test seropositive for antibodies to PPRV without necessarily showing clinical signs.

Detection of nucleic acids

Total nucleic acids were extracted from nasal swabs using QIAamp Viral RNA Mini extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted samples were tested for the presence of nucleic acid material of four pathogens using a multiplex qRT-PCR method developed by Settypalli *et al*. (2016). Briefly, a 20 µL reaction volume containing reagents from iScript™ Universal Probes One-Step Kit (Bio-Rad, Hercules, USA), four pathogen-specific primer pairs (500nM each) and probes (250nM each) labeled at the 5'ends with different reporter dyes: *Capripoxvirus* with Cy5, PPRV with HEX, *P. multocida* with FAM and Mccp with Texas Red. The assay was run using CFX 96™ real-time PCR machine (Bio-Rad, Hercules, USA) with the following cycling conditions: 50°C for 20 minutes followed by 95°C for 5

minutes and 40 cycles of denaturation at 94°C for 10 secs, annealing at 56°C for 20 secs, and extension at 62°C for 20 secs. The data acquisition was performed during the annealing step. An amplification peak and quantification cycle (Cq) value of less 35 was interpreted to indicate presence of a pathogen.

Data analysis

Data generated were entered in Microsoft Excel 2016 and analyzed using descriptive statistics to determine proportion of sheep and goats, sex and age of animal testing positive for PPRV. True prevalence was calculated based on apparent prevalence, and the sensitivity and specificity of the diagnostic test used as described by Rogan and Gladen (1978). The odds ratio was calculated to assess the association between being seropositive for PPR (Altman, 1991) with the value of $p < 0.05$ considered significant. Other factors considered for being seropositive for PPRV were sex and age.

Appendix 2: PPR sample collection questionnaire

A. GENERAL INFORMATION

Consent given? (1=Yes, 2=No) _____
If consent is denied, continue with this questionnaire at another household that agrees to participate.

Name of interviewer	
Date of interview:	
Region	
District	
Ward	
Village	

B. BIO-DATA, HOUSEHOLD/FARM INFORMATION

Head of household's name (optional)	
Respondent's name (optional)	
Respondent's age	
Respondent's sex	o F o M
Respondent's relationship to Household head	o Household head o Spouse o Child o Other family member specify _____ o Other non-family member specify _____
Education level of respondent:	o None o Primary o Secondary o Higher education (tertiary)
Type of farming	o Household o Meat production o Dairy production o Trader
How many goats and sheep do you keep?	o Goats_____ o Sheep_____
What other animals do you keep?	o Cattle o Pigs o Poultry o Others: specify_____
Grazing system	o Private land o Communal o Zero grazing
If zero grazing, where are the animals	

kept?	
What are the animals fed?	
How often do you clean the animal housing facilities?	o Daily o Weekly o Rarely o Never
Who works on the farm?	o Family labour o Hired labour o Both
Do you grow any crops?	o Yes o No
List them, if any? Ignore if No	

C. GENERAL ANIMAL HEALTH STATUS

Do your goats and sheep mix up on the farm in any way with other animals?	o Yes o No
Do your goats and sheep mix up with other herds?	o Yes o No
Do you separate the healthy animals from the sick ones and those receiving therapy?	o Yes o No
Is the animal feed/pasture easily available all year round?	o Yes o No
If no, what action is taken to avail food to the animals (write all that apply)	
Where do you get the water for the animals from?	o Piped water o Communal drinking points o Well water o Borehole o Rainwater o Others; specify _____
Do you keep health records for your animals?	o Yes o No
If not, give your reasons	
What is contained in the health records? Tick all that apply	o Treatment dates o Animal identification o Dosages o Routes of administration o Individual who administered the drug o Drug used

	o Duration of treatment therapy
Are your sheep and goats vaccinated against diseases?	o Yes o No o Some of them o I don't know
If you answered yes above, name the diseases If you answered no above, give your reasons.	o o o It's not important o Service is unavailable o Don't know what vaccination is o I don't want to vaccinate the animals o Other; specify _____ _____
Who diagnoses, treats and administers the drugs to sick animals?	o The farm hands o Traditional healer o Veterinary practitioners and animal health scientists o Others; specify _____ o I don't know
What do you use to treat your sick animals?	o Don't know o Veterinary drugs only o Herbal remedies only o A mixture of both vet drugs and herbal remedies
How many of your animals died in the past 6 months?	o Goat_____ o Sheep _____
What signs of illness were observed prior to death?	
How do you dispose of the carcasses?	
What signs of illness have your animals suffered from in the last 12 months? (Tick all that apply).	o Weight loss o Nasal/ocular discharges o High temperatures o Diarrhoea o Weakness o Reduced milk production o Coughing o Laboured breathing o Foetal abortions o Low appetite o Oral lesions o Unspecified diseases o Others: mention _____ _____

D. KNOWLEDGE AND ATTITUDES ABOUT PPR

Have you heard of PPR before?	o Yes o No
Where did you get information about PPR from?	o From the veterinary officer o The radio o Newspaper o Other farmers o Government offices o Other; specify _____ _____ _____
Have your animals been vaccinated against PPR?	o Yes o No
Have your animals suffered from PPR	o Yes o No
If you answered yes above, what course of action did you take?	o Culled them o Treated them o Sold them o Did nothing o I don't know
When did the outbreak occur?	
How many times have you had the outbreak in the past 12 months?	
Mention some signs of PPR observed during the outbreak	
Did you report the outbreak of the disease to the veterinary officer?	o Yes o No
If yes, what action was taken?	
Did you inform other neighbouring farms about the disease?	o Yes o No
If yes, what action was taken?	