Epidemiology of Peste des Petits Ruminants (PPR) in the High Risk Areas of Muchinga, Northern and North-Western Provinces of Zambia

BY

MALAMA MUMBA

A dissertation submitted to the University of Zambia in partial fulfillment of the requirements for the award of the degree of Master of Science in One Health Analytical Epidemiology

THE UNIVERSITY OF ZAMBIA

DECLARATION

I, Malama Mumba, do hereby declare that this dissertation, submitted by me to the University of Zambia for the degree of Master of Science in One Health Analytical Epidemiology has not been submitted at any other university.

Student name: ……

Date: ……..……

CERTIFICATE OF APPROVAL

The dissertation of Malama Mumba has been approved as fulfilling the partial requirements for the award of Master of Science in One Health Analytical Epidemiology by the University of Zambia.

Dr. N. Saasa	…….	……..
(Supervisor)	Signature	Date
Examiner 1	…….	…….
	Signature	Date
Examiner 2	……	……
	Signature	Date
Examiner 3	……	……
	Signature	Date
Chairman	……	……...
(Board of Examiners)	Signature	Date

ABSTRACT

Peste des Petits Ruminants (PPR) is a highly infectious epizootic disease of small ruminants that cause high mortality and has become an increasingly important trans-boundary disease following the eradication of Rinderpest. PPR is endemic in Africa, Middle East and Asian countries and is a major contributor to poverty in the rural pastoral communities. This exacerbates poverty levels among the smallholder farmers in rural areas who solely rely on small ruminants as a source of income. The disease also causes an economic and social disaster, and ultimately is a threat to the national food security.

Therefore, a study was carried out to determine the epidemiological status of PPR in selected high risk areas of Zambia. A total of 532 serum samples were collected from goats and sheep in Nakonde district in Muchinga Province, Mbala and Mpulungu in Northern Province and Solwezi in North-western Province. These samples were screened for PPR specific antibodies using PPR cELISA. In addition, questionnaires were administered to 31 farmers and veterinary staff in the districts to collect information on risk factors associated with the presence of the disease. To determine the levels of association of risk factors to disease presence, Chi square and multivariable logistic regression were performed in SPSS at 95% confidence level.

Results revealed that PPR was present in all the distrcits. At individual level, 1.70% (9/532) of animals sampled were positive for PPRV antibodies. This represented a district proportion of 0.50 % in Mbala, 1.80%) in Mpulungu, 2.40% in Nakonde and 4% for Solwezi. Univariant analysis of the potential risk factors found trade (p<0.001) and regulation on animal movement (p<0.001) to be important in the epidemiology of PPR However, sex of the animal (p=1.000), age (p=0.092), PPR awareness (p=0.294), veterinary clearance (p=0.062) grazing system (p=0.110) and disease reporting (p=0.099) were not significant. However, multivariable logistic regression showed that PPR awareness (p=0.006) and disease reporting (p<0.000) were also significant.

The apparent absence of overt clinical presentation of the disease in these animals may have been due to failure to diagnose the disease by the farmers and veterinary staff. There is need to determine the seroprevalence of PPR in the studied districts andother districts bordering

Tanzania, DR Congo and Angola. There is need of confining the disease through initiation of surveillance networks, focused ‘ring’ vaccination and animal movement restriction to other parts of the country. The farmers and Veterinary staff in the high risk areas need to be educated on PPR. Also, there is need to strengthen veterinary extension services through deployment of Veterinary staff in camps that did not have staff .

DEDICATION

This work is dedicated to my parents, Rev. Alex C. Mumba and Mrs. Mary Kunda Mumba for educating me and making the best out of me; to my loving husband, Mr. Peter Kaluba for the support and understanding and my children Chibusa and Ng'andwe for putting up with my absence from home.

ACKNOWLEDGEMENT

I wish to acknowledge the Southern African Center for Infectious Disease Surveillance (SACIDS) for sponsoring my study for the degree of Master of Science in One Health Analytical Epidemiology (OHAE) at the University of Zambia (UNZA).

I am also grateful to the Ministry of Agriculture and Livestock (MAL) for granting me the opportunity to pursue my MSc at UNZA. I would like to thank Dr. Paul Fandamu (Chief Veterinary Research Officer at Central Veterinary Research Institute) for encouraging me to apply and study for an MSc degree in Epidemiology.

I would like to thank Dr. Ngonda Saasa, my principal supervisor for his guidance, interest, and support from the time of proposal formulation to the time of completion and submission of this work. I am further grateful to my Co-Supervisors Dr. King Nalubamba and Dr. Martin Simuunza from UNZA who equally played important roles in guiding me on my research work and ensured that I completed my studies in time. Dr. George Dautu and Dr. Gregory Bwalya are equally acknowledged for their invaluable contributions towards this work.

I acknowledge Mr. Mooya and Mr. Witola for the help rendered in the processing of the samples at Central Veterinary Research Institute (CVRI) Virology Laboratory.

I am grateful to Dr. Mundia, Dr. Joseph Banda, Dr. Chalilunda, and Dr. Manley Mazhila the District Veterinary Officers for Nakonde, Mbala, Mpulungu and Solwezi respectively, for facilitating my research work in their districts.

Lastly, I would like to acknowledge my husband Mr. Kaluba for always being there for me, for the support and assistance.

TABLE OF CONTENTS

DECLARATION i

CERTIFICATE OF APPROVAL ii

ABSTRACT iii

DEDICATION v

ACKNOWLEDGEMENT vi

LIST OF FIGURES x

LIST OF TABLES xi

LIST OF ABBREVIATIONS AND ACRONYMS xii

CHAPTER ONE 1

1.0 Introduction 1

1.1 Statement of the Problem and Study Justification 2

1.2 Objectives 3

1.2.1 Main objective 3

1.2.2 Specific objectives 3

CHAPTER TWO 4

2.0 LITERATURE REVIEW 4

2.1 Description of PPR 4

2.2 Causative agent of PPR 4

2.3 History and World distribution of PPR 4

2.4 Distribution of PPRV according to molecular diversity 6

2.5 PPR outbreaks in southern africa 7

2.6 Host range and susceptibility to PPR 8

2.7 Transmission 8

2.8 Infection process and immunity 9

2.9 Clinical manifestation 9
2.10 Diagnosis 10
2.10.1 Clinical and differential diagnosis 10
2.10.2 Laboratory diagnosis 11
2.11 Control measures 13
2.11.1. Surveillance 13
2.11.2 Biosecurity 13
2.11.3 Vaccination 13
2.11.4 Stamping out 14
2.11.5 Public awareness 14
2.11.6 Treatment 15
2.12 Economic impact of PPR 15
CHAPTER THREE 17
3.0 MATERIALS AND METHODS 17
3.1 Study area and livestock census 17
3.2 Study design 18
3.3 Sample size determination 18
3.4 Organisation of the district veterinary structure 19
3. 5 Data collection and tools 19
3.5.1 Questionnaire survey 20
3.5.2 Serum sample collection and storage 20
3.6 cELISA protocol 20
3.7 Data analysis 21
CHAPTER FOUR 22
4.0 RESULTS 22
4.1 Descriptive results 22
4.1.1 Organisation of the District veterinary structure 22

4.1.2 Source of livelihood 22
4.1.3 Livestock population 23
4.1.4 Source of livestock for breeding 23
4.2 Serology 24
4.3 Risk factors 26
4.3.1 Veterinary clearance, livestock trading and awareness on PPR 26
4.3.2 Grazing system 27
4.3.3 Disease reporting 27
4.3.4 Regulation on animal movement 28
4.3.5 Association of risk factors to disease presence 28
4.4 Questionnaire response from the Veterinary staff 31
CHAPTER FIVE 32
5.0 DISCUSSION 32
CHAPTER SIX 35
6.0 CONCLUSION AND RECOMMENDATIONS 35
REFERENCES 36
APPENDICES 47

LIST OF FIGURES

Figure 2.1: Map of the world showing progressive spread of PPR since 1942 5

Figure 2.2: Map of the world showing the distribution of the four PPR virus (PPRV) lineages.. 6

Figure 2.3: Map of SADC region infected, high risk, and low risk countries. 7

Figure 2.4: Clinical signs of PPR in goats 10

Figure 3.1: Map of Zambia showing the location of the study area- Mbala, Mpulungu, Nakonde and Solwezi…….……17

Figure 4.1: Farmers source of Livelihood in the study area……22

Figure 4.2: Livestock population by types in the study area……...23

Figure 4.3: Seropositivity of PPR in the different ages……..26

Figure 4.4: Proportion of traders seeking Veterinary clearance in the study area…….....26

Figure 4.5: Levels of PPR awareness among farmers…… 27

Figure 4.6: Farmers reporting of diseases affectig livestock to Veterinary staff in the study areas…….27

LIST OF TABLES

Table 3.1: Goat and sheep populations in the study areas of Muchinga, Northern and North-western provinces of zambia (NALEIC,2013)……18

Table 3.2: Expected and actual number of samples collected from each district.....19

Table 4.1: Staffing levels of Veterinary camps in Mbala, Mpulungu, Nakonde and Solwezi.....22

Table 4.2: Source of animals for breeding and selling points.....24

Table 4.3: Livestock population, number of animals sampled and cELISA results in the study area……...25

Table 4.4: Sex distribution of PPR positive animals…….25

Table 4.5: Risk factors evaluated for the association with the occurrence of PPR presence……29

Table 4.6: Risk factors associated with PPR disease presence using multivariable logistic regression model……30

LIST OF ABBREVIATIONS AND ACRONYMS

CDV	Canine distemper
CVRI	Central Veterinary Research Institute
cELISA	Competitive Enzyme Linked Immunosorbent Assay
CI	Confidence Interval
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Dévelopement
DVO	District Veterinary Officer
DVS	Department of Veterinary Services
EMPRES	Emergency Prevention System for Animal Health
FAO	Food and Agricultural Oganisation
GIS	Geographical Information System
GRZ	Government of the Republic of Zambia
GPS	Geographical Positioning System
MAL	Ministry of Agriculture and Livestock
mAb	Monoclocal antibodies
NALEIC	National Livestock Epidemiological Information Center
OIE	World Organisation for Animal Health
PPR	Peste des Petits Ruminants
PPRV	Peste des Petits Ruminants Virus
PCR	Polymerase chain reaction
RT-PCR	Reverse transcription polymerase chain reaction
RNA	Ribonucleic acid
RPV	Rinderpest virus
SACIDS	Southern African Center for Infectious Disease Surveillance
SADC	Southern African Development Community
S/N%	Competition percent
TADS	Trans-boundry Animal Diseases
VA	Veterinary Assistant
VNT	Virus neutralisation test
WHO	World Health Organisation

CHAPTER ONE

1.0 Introduction

Livestock production is an important agricultural activity in most of the countries in Africa with goats playing an important socioeconomic role in rural areas. Small ruminants, mainly sheep and goats, play an important role in the livelihoods and food security of poor families (Kitching, 1988; Banyard *et al.,* 2010). This is because goats and sheep are prolific breeders and require low capital inputs for a moderate level of production, reaching maturity early and are profitable. Small ruminants are a source of milk, meat, milk,meat products and income (mobile bank). Also, these small ruminants have a role in returning nutrients to the soil through the production of manure for use in cropping systems. Sheep and goats also play a critical role in the livelihoods of the traders who buy the animals and bring them to urban centres. (FAO, 2011, Steinfeld *et al.,* 2006).

In Zambia, the livestock sector is economically important accounting for about 42% of the total national agricultural gross domestic production. It also shows that livestock contributes about a high share of 45% of income to the poorest households. The livestock population is estimated at 3.2 million cattle, 0.5 million sheep, 0.8 million goats, 33 million poultry, and 110,000 sows. pigs (Anon, 2012). One of the major factors restricting maximum efficient livestock production is the presence of high negative impact of infectious animal diseases. One such disease is Peste des Petits Ruminants (PPR) which is becoming endemic in some of the affected countries in Africa and Asia. PPR is one of the epizootic diseases of small ruminants and is similar to Rinderpest (RPV) of cattle (Anderson *et al.,* 1990; Couacy-Hyman *et al.,* 1995). It is highly infectious and causes high mortality and has become an increasingly important trans-boundary disease following the eradication of Rinderpest (Elsawalhy *et al.,* 2010). Several authors have recognized PPR as one of the major factors limiting small ruminants production in a wide range of agro-climatic zones (Sumberg and Mack, 1985, Kusiluka and Kambarage 1996). Therefore, this disease contributes to impeding safe trade and depriving poor farmers of access to lucrative global markets in livestock and livestock products. This is a serious problem in the developing world, where capacity to cope with the cost and logistics of controlling such diseases is often limited.

Peste des Petits Ruminants (PPR) was officially reported to have affected the Northern part Tanzania in 2008 (Swai *et al.,* 2009) and eventually spread to the south in 2010 (FAO 2010; Muse *et al.,* 2012) thus affecting one of the countries in the Southern African Development Community (SADC) region. Peste des Petits ruminants outbreak was also reported in the Democratic Republic of Congo (DRC) and Angola (FAO 2013). Therefore, PPR is a disease

of increasing importance in Africa, particularly in areas where small ruminants form an important component of agricultural food production such as Zambia.

In Zambia, PPR has not been reported despite sharing borders with the affected countries. Chazya *et al*. (2014) found three districts comprising of Nakonde, Mpulungu and Mbala to have a high risk of occurrence of PPR. However, such conclusions did not focus on determining the presence of PPR in the high risk areas of Zambia. It is against this background that this study investigated the seropresence of PPR in the high risk prone areas of Northern and North-western parts of Zambia.

1.1 Statement of the Problem and Study Justification

PPR is a disease that threatens the national food security of affected countries due to the high morbidity and mortality rates and has significant economic impacts in developing countries (Wambura 2000). The SADC region was spared from PPR until the recent infectios in Tanzania, DRC and Angola (Swai *et al.,* 2009; OIE, 2012b; SADC, 2012; FAO, 2013a;). In Zambia, PPR is a notifiable disease which has not been reported despite the country sharing the boarders with the affected countries.(SADC, 2012a). With the infection of the these countries in the region, countries like Zambia, Malawi and Mozambique which shares borders with them are at immediate risk (SADC, 2012b) Previous findings established by Chazya *et al.,* (2014) found that there was a high risk of occurrence of PPR through the importation of PPR infected live animals from Tanzania. The principal mode of transmission for the PPR is through movement of infected livestock, most of which is illegal (Muse *et al.,* 2012; FAO, 2013). PPR is easily transmitted by direct contact between live animals in shared pastures and at live animal markets (Lefevre and Diallo, 1990). Despite these findings, trade in livestock and livestock products has continued to exist putting Zambia at a risk of having the disease. No study has been carried out to investigate its presence in the high risk areas. Once the virus is introduced into the high risk areas of Zambia, the rest of the county could easily get affected considering the limited disease surveillance efforts and inadequate veterinary service delivery. Therefore, there was need carry out the study to determine the presence of PPR in the high risk prone areas of Northern, Muchinga and North-western parts of Zambia. It is of great importance that once the presence of PPR is established, the findings will be used as a basis for recommendations to the policy makers to help in policy formulation in controlling the disease, improve surveillance system and animal health.

1.2 Objectives

1.2.1 Main objective

To evaluate the epidemiological status of PPR in small ruminants in in the high risk areas of Muchinga, Northern and North-Western provinces of Zambia.

1.2.2 Specific objectives

a) To determine the seropresence of PPR in areas bordering Tanzania and Democratic Republic of Congo (DRC).

b) To assess the levels of PPR awareness and risk factors associated with PPR among small ruminant farmers.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of PPR

Peste des Petits Ruminants (PPR) is an acute, highly contagious transboundary viral disease of wild and domestic small ruminants also known as 'goat plague' (Barrett *et al.,* 2005; Nussieba *et al.,* 2009; Lefèvre *et al.,* 2010). PPR is considered as one of the main constraints in augmenting the productivity of small ruminants in developing countries as well as severely affects poor farmer's economy (Balamurugan *et al.,* 2013). It is a viral disease characterized by fever, oculonasal discharge, stomatitis, diarrhoea, bronchopneumonia, and sometimes death. Although cattle and several wild ruminants have often been infected, goats and sheep are the natural targets. PPR is clinically similar disease to rinderpest caused by rinderpest virus (RPV) of cattle (Diallo *et al.,* 2007). Although, the natural disease affects mainly goats and sheep, goats suffer more severe clinical disease. (Raghavendra *et al.,* 2000; Khan *et al.,* 2008).

2.2 Causative agent of PPR

Peste des Petits Ruminants (PPR) is caused by a Peste des Petits Ruminants virus (PPRV), an RNA virus belonging to the genus *Morbillivirus* and family *Paramyxoviridae* (Gibbs *et al.,* 1979; Murphy *et al.,* 1999). Morbilliviruses are highly contagious pathogens that cause some of the most devastating diseases of humans and animals worldwide (Murphy and Parks, 1999).This genus also includes six viruses: Measles virus (MV), Rinderpest virus (RPV), Canine distemper virus (CDV), Phocid morbilliviruses (PMV) and Porpoise distemper virus (PDV) (Domingo *et al.,* 1990, McCullough *et al.,* 1991).. These viruses share the same genome organization although their ribonucleic acid (RNA) lengths differ slightly (Barrett *et al.,* 1993; Barrett, 2001). Morbilliviruses display the typical structure of *Paramyxoviridae:* a pleomorphic particle with a lipid envelope, which encloses a helical nucleocapsid (Gibbs *et al.,* 1979). Morbilliviruses are linear, non-segmented, single stranded, pleomorphic, negative sense RNA viruses with genomes 15-16 kilo bases in length and 200nm in diameter (Norrby and Oxman, 1990; Libeau *et al.,* 1995). PPR is one of the longest sequenced morbillivirus genomes comprising of 16 kilo base pairs (Bailey *et al.,* 2005).

2.3 History and World distribution of PPR

For a long time, the PPR virus was assumed to be a variant of rinderpest (Diallo, 1988). Recent development of specific and sensitive molecular and serological techniques made genetic, antigenic and serologic differentiatiation between RPV and PPR easy and rapid Balamurugan *et al.,* 2013). The development of monoclonal antibodies (mAbs) based

enzyme linked immune-sorbent assay (ELISA), specific nucleic acid probes for hybridization studies and nucleic acid sequencing confirmed that PPR virus is fairly distinct from RPV (Taylor, 1979; Diallo *et al.*, 1989; Diallo *et al*., 1995).

The geographical distribution of PPR has steadily expanded to cover large regions in Africa, the Middle East and Asia (Fig 2.1). PPR was first described in Ivory Cost of West Africa in 1942 (Gargadennec and Lalanne 1942). Overtime,the disease was subsequently confirmed in Nigeria, Senegal and Ghana. Further studies have isolated the PPR virus in Nigeria, Saudi Arabia, India and Turkey. Earlier, the disease was thought to be restricted to the western part of Africa until goats were affected with the disease in Sudan (El Hag and Taylor, 1984), which was originally diagnosed as rinderpest in 1972 but later confirmed to be PPR (Diallo, 1988). Serological evidence was detected in Syria, Niger and Jordan while the presence of the virus was confirmed in Ethiopia and Eritrea. In the SADC region Tanzania, DRC and Angola are affected thus putting Zambia at risk of getting the disease.

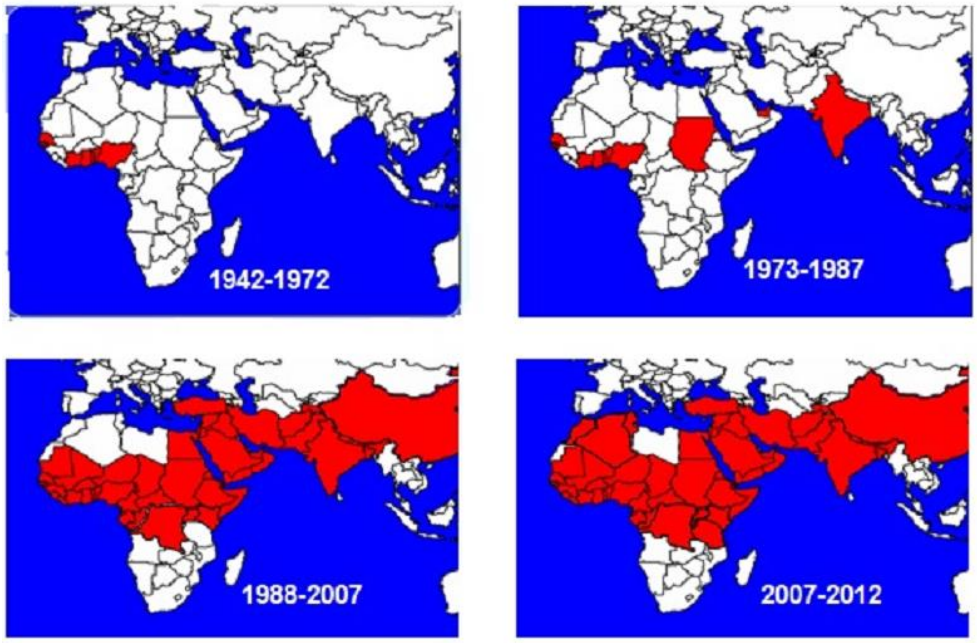

Figure 2.1: Map showing progressive spread of PPR in the world since 1942 when it was first reported. PPR outbreak areas are marked in red and white are free areas (FAO, 2012)

Shaila *et al.* (1996) suggests that probably, many of the cases diagnosed as rinderpest among small ruminants may, instead, have been PPR. This is supported by the emergence PPR and its spread to around 70 countries in Africa, the Middle East and Asia indicating its growing importance (OIE & FAO, 2015). These regions are a home to over 80 percent of the world's sheep and goats and to more than 330 million of the world's poorest people who depend on small ruminants as a source of livelihood. Because of PPR's widespread occurrence (Couacy-Hymann *et al.*, 2002) and the devastating economic impact it causes, the disease has received

a rising attention (Lefèvre and Diallo, 1990, EMPRES, 2009; FAO, 2012; OIE & FAO, 2015).

2.4 Distribution of PPRV according to molecular diversity

The current molecular characterization of PPRV isolates them into four genetically distinct lineages (Figure 2.2): lineage I being represented mainly 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 East Africa, from Sudan, Yemen and Oman; while lineage IV includes all viruses isolated from recent outbreaks across the Arabian Peninsula, the Middle East, southern Asia, and recently, across several African territories (Banyard *et al.*, 2010). Constant increase of the disease incidence has been associated with this lineage, particularly that which occurred in North and East Africa and in Asia, from 2004 to 2013. This suggests an increase in virulence (Kwiatek *et al.*, 2011). In addition, countries otherwise known to have regular epizootic activity such as Sudan, Central African Republic and Cameroon, proved to harbour lineage IV. Currently, in a large zone encompassing Sudan, Ethiopia, Somalia and Kenya, lineage IV is slowly replacing PPRV lineage III (Khalafalla *et al.*, 2010; Kwiatek *et al.*, 2011). The presence of the two African lineages in Asia beside a distinct Asian lineage indicates trade as a factor for the disease spreading.

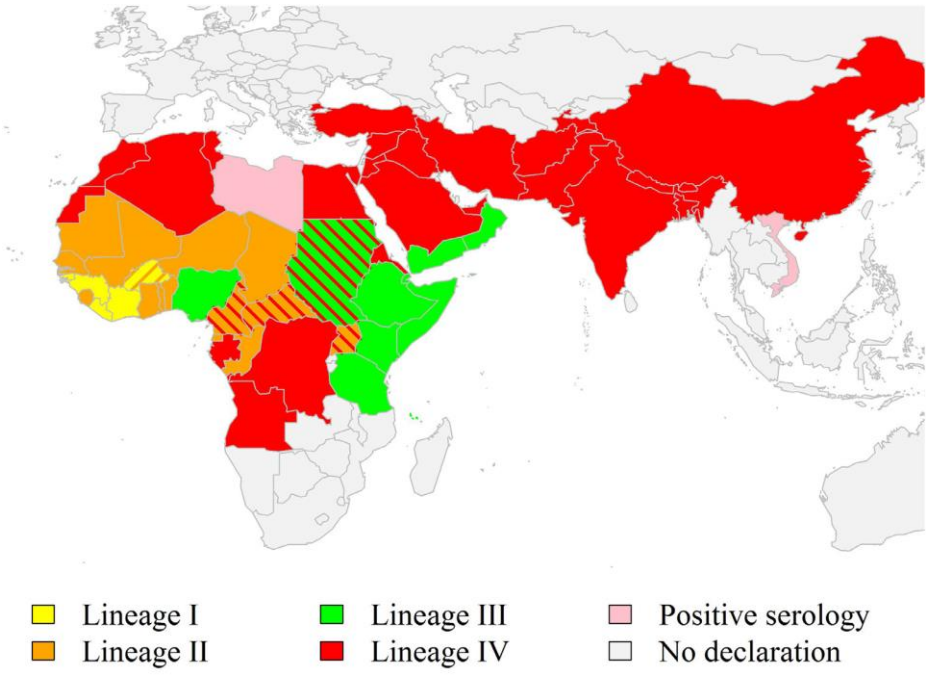

Figure 2.2: Map depicting the distribution of the four PPR virus (PPRV) lineages. Countries reporting at least one PPRV lineage are colored according to the identified lineages. Background color and colored bars represent the last identified lineages in the country (Albina et al. 2013).

2.5 PPR outbreaks in southern africa

PPR is currently believed to be endemic across much of West Africa. Historically, it was hypothesized that PPRV spread into North and East Africa from West Africa, moving up through trade routes. PPR has continued to spread from North Africa (Algeria, Tunisia, Morocco) to the southern part reaching as far as Tanzania. More recently, PPR has affected three countries in the SADC region namely Tanzania, DRC and Angola (Figure 2.3) (Kivaria *et al.*, 2009; Swai *et al.*, 2009; FAO 2010; FAO 2013a; Kivaria *et al.*, 2013). According to Muse *et al.* (2011), trading dynamics in small ruminnats between Zambia and the PPR affected SADC countries presents a great risk.

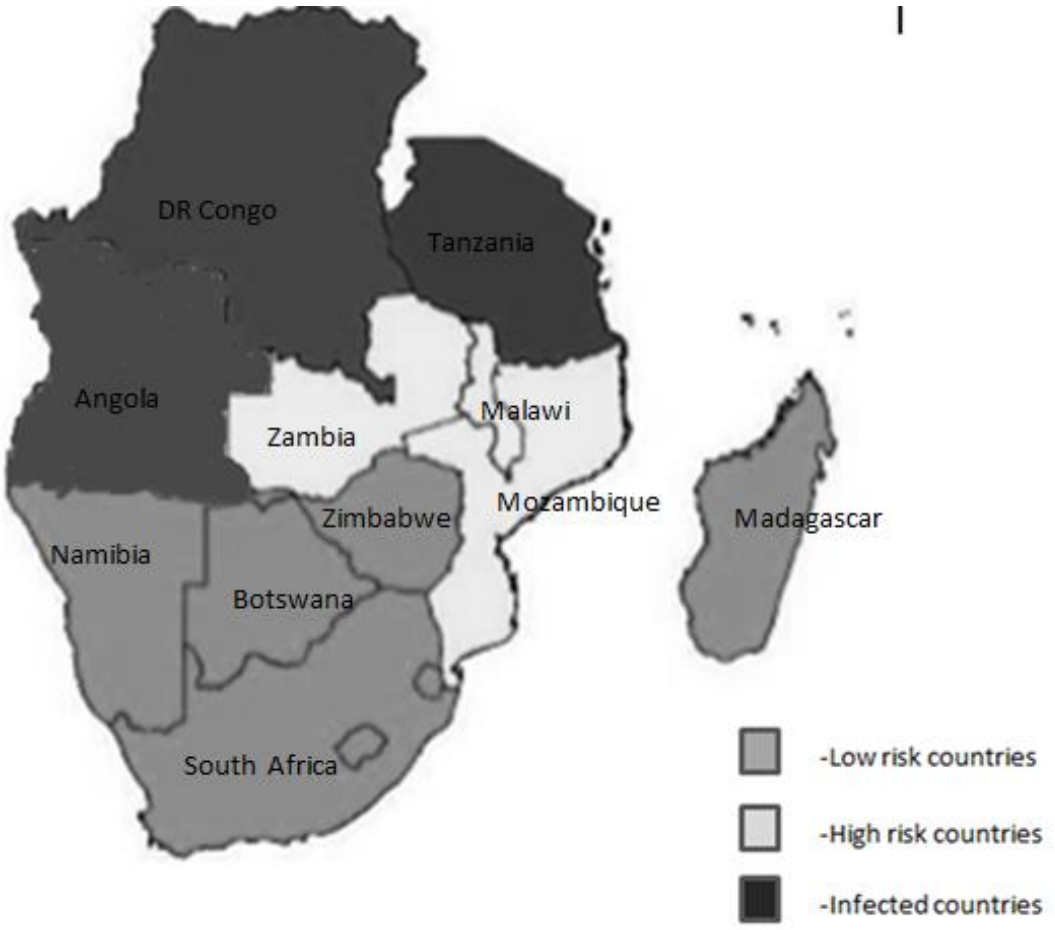

Figure 2.3: Map of SADC region as per PPR risk classification showing; infected, high risk, and low risk countries (sadc, 2012).

2.6 Host range and susceptibility to PPR

PPRV generally causes an acute disease in small ruminants. Sheep and goats are the primary hosts, although sheep are often less severely affected (Lefèvre and Diallo, 1990; Khan *et al.,* 2008). Cattle, buffalo and pig can be infected and may show clinical signs. The disease has an immunosuppressive effect in affected animals which can lead to secondary infections. Similarly, Diallo *et al.* (2007) has reported large ruminants such as cattle harbour the virus and are able to transmit it to sheep and goats. Several authors have reported the disease in Gazelle, Deer, Antelope and other small wild ruminant species (Abu Elzein *et al.,* 2004). It has now been shown that camels are susceptible to the PPRV and that the clinical expression of the disease is emerging (Khalafalla *et al.,* 2010; Kwiatek *et al.,* 2011). Kinne *et al.* (2010) has further reported the existence of sylvatic reservoirs for PPRV with infections and deaths in captive wild ungulates.

Newborn animals become susceptible to PPRV infection at three to four months of age (Srinivas and Gopal 1996). This corresponds with the natural decline in maternal antibodies (Saliki *et al.,* 1993). Moreover, it is this large number of young susceptible stock that may also account for the severity of PPRV infection in goat population. However, other research done in Saudi Arabia found a morbidity and mortality in all age groups of 90% and 70% respectively (Abu Elzein *et al.,* 1990). Serological evidence revealed that antibodies occur in all age groups from 4-24 months indicating a constant circulation of the virus (Taylor, 1979).

.

2.7 Transmission

PPRV is transmitted through direct contact between infected and susceptible animals. Wild animals may often come into contact with local sheep and goat populations from whom they contract the virus (Shankar *et al.,* 1998). Similarly, infected wild animals may transmit the virus to susceptible sheep and goat population. Muse *et al.* (2012) found that the purchase of PPR potentially infected animals and their subsequent introduction into naïve flocks/herds further spread the disease. The disease is transmitted by aerosols between animals living in close contact (Lefevre and Diallo, 1990). Discharge from eyes, nose and mouth, as well as the loose faeces, contain large amounts of infective virus. Therefore, close contact between live animals in shared pastures and at live animal markets has led to movement of infected livestock as the principal mode of transmission for PPR. Meanwhile, contaminated water, feed troughs, and bedding act as a form of indirect transmission, however the virus does not survive for a long time outside the body of a host animal.

2.8 Infection process and immunity

The virus enters via the respiratory tract. The first replication starts in the retropharyngeal and mandibular lymph nodes and tonsils. After 2-3 days, viraemia may develop, and 1-2 days later, the first clinical signs may appear. Since animals excrete the virus before showing signs of the disease, it can spread by movement of infected animals. The virus is disseminated to a variety of organs like lymph nodes, bone marrow, spleen, mucosa of the digestive tract, and the upper and lower respiratory tract (Chauhan *et al.*, 2009).

The protective immune response is usually elicited against the surface F and H proteins of PPRV (Diallo *et al.*, 2007). However, among the viral proteins, most of the neutralizing antibodies are directed against the H protein during PPRV infection (Diallo *et al.*, 2007). In all members of the genus Morbillivirus including PPRV, the N protein is the most abundant viral protein due to its presence at the extreme 3´-end of the viral genome. Owing to its high quantity during infection, the N protein is considered the most immunogenic, but the immunity produced against N protein does not protect the animals from the disease. By virtue of the nature of the H and N proteins, these remain the most acceptable targets for the design of PPRV diagnostic tools (Munir, 2011).

2.9 Clinical manifestation

PPR is characterized by fever followed by watery oculo-nasal discharge, gradually becoming mucopurulent and stick parts of the eyelids together. Several authors have extensively documented the clinical signs of PPR (Hamdy *et al*., 1976; Taylor, 1984; Roeder *et al*., 1994; Roeder and Obi, 1999).

Following infection there is a 3 - 4 days incubation period. During this period, the virus replicates in the draining lymph nodes of the oro-pharynx before spreading via the blood and lymph to other tissues and organs including the lungs causing a primary viral pneumonia. The affected animals are characterized by sudden rise in body temperature to 39.5 - 41°C and tachypnoea. Also the infected animal experiences difficulty and noisy breathing marked by extension of the head and neck indicating obvious signs of pneumonia (Taylor, 1984; Radostits *et al.,* 2007). Other symptoms includes dilation of the nostrils, protrusion of the tongue and coughing. A clear watery discharge is observed from the eyes, nose and mouth, later becoming thick and yellow as a result of secondary bacterial infection. Appearance of a serous to mucopurulent nasal discharge which may crust over and occlude the nostril (Figure 2.4) ocular discharge resulting in matting of the eyelids. One to two days after fever has set in, the mucous membranes of the mouth and eyes become reddened. Then, epithelial necrosis causes small pin-point greyish areas on the gums, dental pad, palate, lips, inner aspects of the

cheeks and upper surface of the tongue (Figure 2.4). These areas increase in number and size and join together. Body temperature usually remains high for about 5-8 days, and then slowly returns to normal prior to recovery or drops below normal before death.

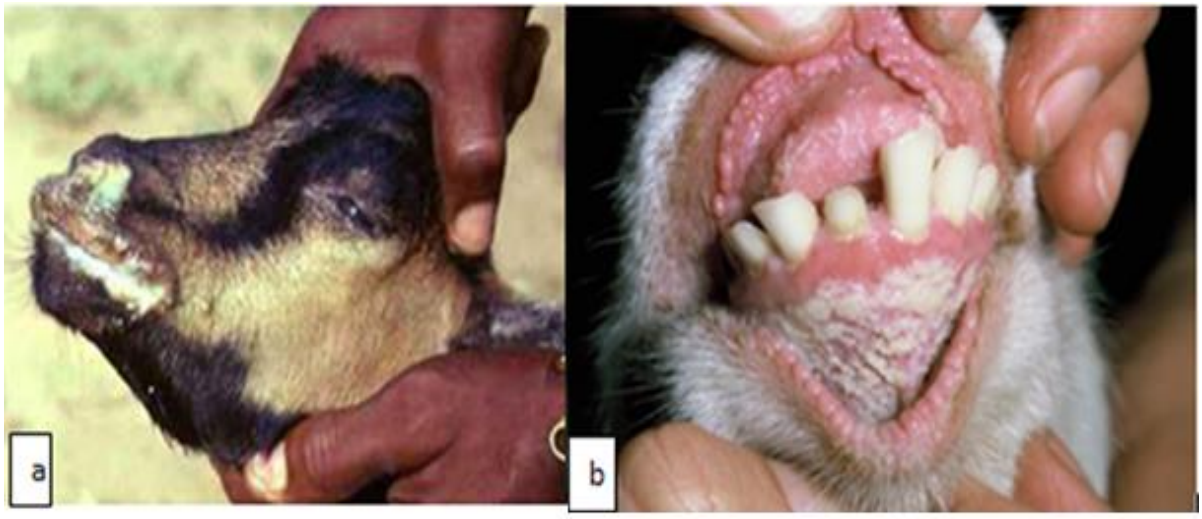

Figure 2.4: (a-b): Clinical signs of ppr in goats; (a) mucopurulent nasal and mouth discharge and (b) ; erosive stomatitis with dead cells on the gums involving the inside of the lower lip (b) (roeder and obi, 1999)

2.10 Diagnosis

Epidemiological features should be considered together with clinical signs to help diagnose PPR. Sheep and goats may be infected with both rinderpest and PPR. Clinical differential diagnosis may not be possible as both viruses in small ruminants produce similar disease. Therefore, tentative clinical diagnosis may have to be confirmed by laboratory analysis. Diagnosis of PPR may be performed by virus isolation, detection of viral antigens, and nucleic acid sequencing and detection of specific antibody in serum. The diagnostic tests employed to confirm PPR depends upon level, skill and resources of the laboratory. Rinderpest and PPR antibodies might be distinguished by either cross virus serum neutralization tests and cELISA using monoclonal antibodies (Anderson and McKay, 1994).

2.10.1 Clinical and differential diagnosis

The typical clinical form is characterized by high fever, depression and anorexia, followed by ocular and nasal discharge, erosive stomatitis, pneumonia and severe diarrhoea. High morbidity and mortality rates of up to 80 to 90 % in naive affected herds make PPR an important killer disease for small ruminant populations. PPR can be confused with diseases such as rinderpest, pastuerellosis, contagious ecthyma, contagious caprine pleuropneumonia (CCPP), bluetongue and heartwater.

2.10.2 Laboratory diagnosis

For a definitive diagnosis, the virus or specific antigen or antibodies need to be demonstrated. The recommended specimens from live animals are swabs of conjunctival discharges, nasal secretions, buccal and rectal mucosae, and anticoagulant-treated blood (OIE, 2013). Prompt detection of PPRV in the field is important for effective PPR control, thereby reducing the potentially serious economic damage which can result from an outbreak. The following laboratory tests are used for the definitive diagnosis of PPR.

2.10.2.1 Serology

For specific diagnosis of PPR in small ruminants, it was essential to differentiate it clearly from the RP. Conventional serological tests like agar-gel immunodiffusion (AGID), Counterimmunoelectrophoresis (CIEP) etc. often fail to differentiate between them. Conventional serological tests like AGID, CIEP etc. often fail to differentiate between them The immunity elicited by the different viral proteins have led to the manufacturing of the H or N-based cELISA. Monoclonal antibodies raised against hemagglutinin protein of PPR virus which have been used either in a blocking ELISA (Aslam *et al.,* 2009; Saliki *et al.,* 1993) or in a cELISA ((Lefevre, *et al.*, 1991; Anderson and McKay, 1994) for differentiation of PPRV and RPV antibodies. Alternatively, nucleoprotein or recombinant antigen has also been used for developing ELISA for specific detection of RP and PPR antibodies (Libeau et al., 1995). The N-monoclonal antibody (N-Mab) based cELISA detect the natural anti-N antibodies which are not protective but are produced in large quantities and are excellent markers of infection or vaccination for serological diagnosis. The recent development of monoclonal antibody (mAb) based ELISAs has allowed the rapid and simple differential diagnosis of RPV and PPR.

2.10.2.1.1 Competitive Enzyme-linked immunosorbent assay (cELISA)

Competitive ELISA is used for the specific detection of PPR or RP antibodies (Abraham and Berhan, 2001; Diop *et al.,* 2005; Choi *et al.,* 2005; Kivaria *et al.,* 2013). It is the most suitable choice as it is sensitive, specific, reliable, and has a high diagnostic specificity of 99.8% and sensitivity of 90.5% (Abubakar *et al.,* 2008). Specific antibodies in serum Monoclonal antibody-based cELISA has been used for the specific measurement of antibodies to (PPR) viruses in sheep, goats, cattle and buffalo (Khan *et al.,* 2008). In present epidemiological scenario, this cELISA kit could prove to be an important tool for sero-monitoring and serosurveillance of PPRV antibodies.

2.10.2.1.2 Viral neutralisation test (VNT)

The virus neutralization test (VNT) is a gold standard test used to diagnose PPR. The test is able to specifically diagnose PPR in small ruminants as it is able to clearly differentiate PPR from the Rinderpest virus by performing a cross-viral neutralisation. Although, it is sensitive, specific and most reliable test for detection of morbillivirus antibodies, it is laborious, time-consuming and expensive and difficult especially when sample size is large (Anderson and McKay 1994; Singh *et al.,* 2004; Libeau *et al.,* 1992). The standard neutralization test is carried out in tissue cultures of primary lamb kidney cells or Vero cells.

2.10.2.2 Molecular techniques

The use of the nucleic acid directed methods have made important contributions to disease control and have improved national and international trade. These include polymerase chain reaction (PCR), Real time PCR and PCR sequencing which are used as diagnostic and characterization tools in veterinary diseases (Pestana et al., 2010). Molecular techniques such as PCR have emerged as highly specific and sensitive tests, which are also useful in molecular characterization of the virus. Reverse transcription polymerase chain reaction (RT-PCR) is a PCR technique which has been the most popular and highly sensitive tool for diagnosis of PPR. Positive RT-PCR results were still obtained with poorly preserved samples, where AGID and virus isolation failed to detect the virus. RT-PCR also offers the possibility of analyzing the relationship between the different PPRV strains for molecular epidemiological studies (Shaila et al., 1996). However, the required set up of the molecular technology laboratory, the running cost and the training of staff is required in order to have quality assured results.

2.10.2.3 Virus isolation

Isolation of the virus by using cell culture is a sensitive method for diagnosis of PPR. Virus may be isolated during the acute stage of the disease when clinical signs are still apparent. Virus is present for approximately 10 days after the onset of fever. Swabs of the eye (conjunctival sac), nasal secretions, and mouth and rectal linings, as well as clotted and whole blood (with EDTA anticoagulant), may be used for isolation. Lymph node or spleen biopsies may also be considered. Best samples for virus isolation in live animals when samples were taken during high temperature and before diarrhoea has started hyperthermic phase (Lefevre, 1990). Some of thewidely used cell culture systems include primary lamb kidney and Vero cells (Taylor, 1984; Hamdy *et al.,* 1976). Vero cells are however widely used for their

continuity and low liability of contamination. However, cell culture laboratories installation and there maintainance is expensive.Therefore, new technology and research has led to the development of specific and rapid tests for the diagnosis of PPR and RPV.

2.11 Control measures

The development of sheep and goat production and value chains requires stability. Therefore, the elimination of animal diseases in general and transboundary diseases such as PPR in particular should be a priority. This is critical for decision-makers interested in making food value chains less risky for the people involved and the consumers they supply. Measures such as the control and eradication of PPR improves the income from small ruminant husbandry systems, through reducing production costs and thus increasing productivity and profitability. This in turn will allow the small ruminant economy to contribute to the overall economic development of the national economy. There are several methods that are used to prevent and control PPRV infections. These include; surveillance, movement control, vaccination and stamping out.

2.11.1. Surveillance

When analysing the surveillance options to employ, it is important to determine the objectives. Ideally, surveillance should aim to; define the extent of the disease, detect new outbreaks, establish disease free zones, monitor disease trend, and inform decision making at various disease control critical points (SADC, 2012b).

2.11.2 Biosecurity

The main mode of PPR virus transmission is through animal movement, which can either be illegal or under permit. With the advent of free movement of people and goods (including livestock and livestock products) as the SADC region moves ever closer to a borderless region, it is no longer enough for a country to rely on national animal movement controls of its neighbour to stem the spread of transboundary animal diseases (TADs) (SADC, 2012b). When control of animal movement is used as one of the arms of an effective control of TADs, it should be implemented in a very careful and strict manner (Wakhusama et al. 2011). All stakeholders such as the police, customs officials and farmers themselves must be engaged to support the movement restrictions from infected areas.

2.11.3 Vaccination

To halt further spread of the disease, targeted vaccination of small ruminants based on critical control points such as livestock markets and transport routes used by traders and semi pastoralists is recommended. Animals recovered from PPR infection or immunized by

vaccination are not a PPRV carrier and do not play a role in maintaining virus circulation in an area. Animals that are not 'immunized' against the virus through natural infection or vaccination can be subclinically infected and constitute a high risk in maintaining and diffusing the virus without apparent clinical signs (Wakhusama *et al.*, 2011).

The advantages of vaccination as a control option are that the vaccine is readily available and very cheap. It confers immunity which lasts for 3 years and hence most animals will only need two vaccinations in their life time. However, annual vaccination is recommended due to the high reproductive rate of small ruminants. It will be important to determine which strain(s) are circulating in newly infected countries. Control of PPR outbreaks relies on movement control (quarantine) combined with the use of focused (ring) vaccination (Roeder and Obi, 1999).

2.11.4 Stamping out

This option is favoured only in situations where the infected population is small and well defined and government has mechanisms in place to compensate the affected farmers. Stamping-out programs involve the eradication of a disease by the destruction of all infected animals. When outbreaks occur, protection and surveillance zones are established around the outbreak area, and animals in the protected zone are destroyed. The level of surveillance in the area should be increased and the movement of animals from surveillance zone should be restricted. This has the ability to regain previous disease free status quickly and therefore be able to trade again as the biggest advantage (SADC, 2012b).

This option is best suited for high risk areas with low density of animals and for low risk areas such as Zambia. The main disadvantage of this option is that it is usually an expensive and therefore unattractive exercise for the State and as a result there is little political will to implement stamping out. It also has social and economically devastating consequences for affected communities even if they will be compensated. Other disadvantages linked to this option are loss of genetic material, diminishing the national herd andis difficult to carry out in light of lack of fences and zones to curtail movement in the event of an outbreak. In addition politically is very difficult (SADC, 2012b).

2.11.5 Public awareness

There should be need to create awareness through simple technical messages for farmers, traders, politicians, community leaders, the media, law enforcement officers, and the general public at large (SADC, 2012). There is also need to train field staff in the available control options such as vaccination, stamping out, zoning, and biosecurity. The government should

also provide the necessary material and financial resources to implement the available control options.

2.11.6 Treatment

Although there is no specific treatment for PPR, antibiotics and other supportive treatment are normally used to prevent secondary infections and decrease mortality (OIE 2012).

2.12 Economic impact of PPR

Peste des petits ruminants (PPR) causes a staggering USD 1.45 billion to USD 2.1 billion in losses each year (OIE & FAO, 2015). Sheep and goats play an important role in the livelihoods and food security of most smallholder famers. There critical role extends to the livelihoods of the traders who buy the animals and bring them to urban centres. Trading involves the use of transport and is a source of additional employment. People are also involved in running businesses to slaughter animals, dress carcasses and cure skins (FAO, 2011).

The largest category of beneficiaries of sheep and goat production and value chains consists of consumers, both rural and urban. Consumers demand is currently changing, with a trend towards urbanised living and increasing wealth (FAO, 2013). These consumers benefit from access to high quality food products such as milk, dairy products and meat, leather from the skins of the animals and wool and fibre for clothing (OIE & FAO, 2015). As demand rises, there is a need for improved production and supply systems to maintain reasonable prices. Fluctuations in the supply of sheep and goat products can have an impact across society and at specific times can affect the diets of many consumers (OIE & FAO, 2015). In addition to this important economic role, sheep and goats are significant in socio-cultural activities such as funerals, dowries and festivals. They are used as gifts or sacrifices for traditional rituals and religious purposes, such as Eid for Muslims. Sheep and goats can be managed under many production systems and are resilient to drought (Ngategize, 1989; Jones and Thornton, 2009).

PPR has significant economic, food security and livelihood impact on the affected communities (FAO, 2011). The socio-economic significance of PPR is as a result of heavy losses at production level and market effects along the value chain. It is estimated that 10% of the total impact of the disease is on trade and public expenditure and 90% on herd productivity (FAO, 2012). According to a report by FAO (2012), it was found that the flock size and value dropped by 10% . They also found that the ability of small ruminants to sustainably support household livelihood decreased by about 30%. In 979, an outbreak of

PPR in Nigeria led to an estimated loss of US$ 75 million (Wakhusama *et al.*, 2011). In the 2008, Njagi (2009) reported an outbreak in Kenya, that costed €12 million to pursue vaccination campaigns of which estimated of € 4.8 million was allocated towards vaccines. According to FAO (2013a), the disease caused death of about 120,000 small ruminants valued at US$5.3 million from 2010 to June 2012 in the Democratic Republic of Congo (DRC) . The estimate that was done in the DRC did not take into account the socioeconomic impact the disease caused and other benefits of goats and sheep to the smallholder farmers. (SADC, 2012). Other major outbreaks in Turkey and India in recent years have indicated a marked rise in the global incidence of PPR (Ozkul *et al*., 2002). The most important direct economic losses caused by the disease are often aggravated by the sanitary measures imposed by authorities to control animal movement and by trade restrictions on animal by-products. Not only does this severely affect rural economies, but it also reduces genetic resources and endangers breeding policies.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area and livestock census

The study was conducted in Mbala and Mpulungu districts in Northern Province of Zambia, Nakonde district in Muchinga Province and Solwezi district in Northwestern province (Figure 3,1). These districts were purposively selected based on their relative proximity to Tanzania and the Democratic republic of Congo (DRC). The trade in live animals between Zambia and these neighboring countries led to their inclusion in this study. The geographical position of Mbala is latitude 8°50′0″ South and longitude 31°23′0″, while Mpulungu is located at latitude 8°46′0″ South and longitude 31°8′0″ East. Meanwhile Nakonde is located at latitude 9°20′0″ South and longitude 32°46′0″ East with Solwezi at latitude 12°11′0″ South and 26°24′0″ East.

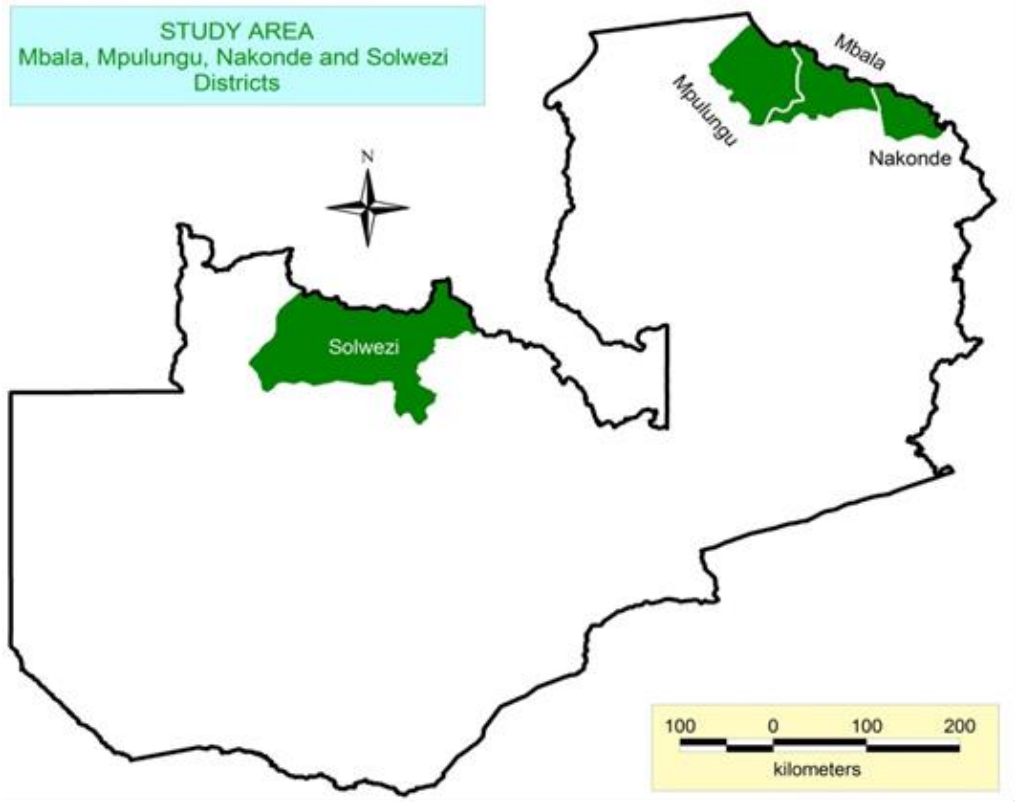

Figure 3.1: Map of Zambia showing the location of the study area- Mbala, Mpulungu, Nakonde and Solwezi districts.(Anon, 2015)

Data on the livestock census for Mbala, Mpulungu, Nakonde and Solwezi were obtained from the National Livestock Epidemiological Inormation Center under (NALEIC) under the Ministry of Agriculture and Livestock (MAL), Department of Veteriary Services (DVS). The total goat and sheep population for the four districts was estimated at 52,992 and 2,733 respectively (Table 3.1).

Table.3.1: Goat and sheep populations in the study area of muchinga, northern and north-western provinces of zambia (naleic, 2013).

District	Goats	Sheep
Mbala	19,420	575
Mpulungu	7,507	506
Nakonde	15,715	798
Solwezi	10,350	854
Total	**52,992**	**2,733**

3.2 Study design

A cross-sectional study was done through May to June 2015 in selected districts of Zambia along the border with Tanzania and DRC. Processing of samples was done at the Central Veterinary Research Institute (CVRI) in Chilanga district.

3.3 Sample size determination

To have statistically amenable results, the required minimum number of samples that were collected in order to detect the presence of disease was calculated according to Thrusfield (1997). The sampling protocol was designed to detect a seroprevalence of 1% and the 95% (0.95) probability was used to detect at least one positive animal. Table 3.2 below shows the expected and actual number of samples that were collected.

$$n= \{1 - (1- p_1)^{1/d}\} (N - d/2) + 1, \text{ where}$$

N=population; d=number of affected animals in the population; n=required sample size; p_1=probability of finding at least one case in the sample.

Table 3.2: Expected and actual number of samples that were collected from each district

District	Goat population	Sheep Population	Expected number of samples	Samples collected
Mbala	19,420	575	297	205
Mpulungu	7,507	506	293	112
Nakonde	15,715	798	296	165
Solwezi	10350	854	294	50
Total	**52,992**	**2,733**	**1180**	**532**

In Mbala and Mpulungu, a stratified random sampling was applied by dividing the study population into exclusive groups called strata based on type of grazing system practiced and shared common water sources. These strata were referred to as villages in this study. Then, a random sampling from all the individual strata was done. While in Nakonde and Solwezi snowballing type of sampling was applied as the sampling frame was not establish. The districts did not have a register of the farmers who were keeping sheep and goats, therefore, unknown farms were identified from the already known farmers and these were recruited to be part of this study.

3.4 Organisation of the district veterinary structure

In Zambia, districts are divided into veterinary camps run by veterinary assistants (VA's) who in turn report to District Veterinary Officers (DVOs). The DVOs are in charge of the districts and they co-ordinate disease control activities in their territories.

3. 5 Data collection and tools

Data was collected through a questionnaire survey and blood samples were collected from sheep and goats.

3.5.1 Questionnaire survey

A structured questionnaire was administerd to each individual household in order to obtain information that would assist in determing risk factors that were associated with the disease presence. In order to have a uniform set of questions for the people in the study area, a semi-structured questionnaire (Appendix 1) was developed and field tested on a few households keeping goats and sheep in the districts. The questionnaire was prepared in English and interviews were conducted using local languages (Bemba, Namwanga) or English where appropriate.The interviews focused on collection of information on flock size, species, age and sex, health , management practices, common diseases in goats and sheep. Other data collected included; movement patterns of livestock in the study areas with the affected neighboring countries, surveillance methods used and knowledge on PPR. The information on the capacity of the Veterinary department to conduct surveillance was collected from the relevant district veterinary offices.

3.5.2 Serum sample collection and storage

Geo-referencing was done on all the farms where samples were collected (appendix 3). Blood was collected by jugular-vein puncture using vacutainer needles and tubes. The blood was transported to the district laboratories where it was left to clot overnight or centrifuged and the serum was decanted into appropriately labeled sterile serum vials. The samples were transported in cooler boxes packed with ice packs to the Central Veterinary Research Institute (CVRI) where they were stored at -20°C until analysis.

3.6 cELISA protocol

PPR cELISA kit is an innovative diagnostics (ID) used for detection of antibodies against the PPR virus in sheep and goat serum and plasma by competitive screening ELISA. The test uses technology developed by the FAO reference laboratory CIRAD-EMVT in Montpellier, France. This diagnostic kit is designed to detect antibodies directed against the nucleoprotein of the PPR virus (Appendix II).

All reagents were allowed to reach room temperature before use. Then, all the reagents were homogenized by vortexing after which 25 µl of dilution buffer 13 was added to each well. Thereafter, a 25 µl each of the positive control, negative control and 25 µl of each sample to be tested were added to individual wells on the testing platet. The plate was incubated at 37 °C for 45 minutes and thereafter, washed 3 times with approximately 300 ul of the wash solution and slap dried on a paper towel while avoiding the drying of the wells between washings. After adding 100 µl of the conjugate to each well, the plate was incubated for another 30 minutes at 21°C. After this incubation, the plate was again washed and slap dried

on a paper towel 3 times with 300 µl of wash solution. Hundred microlitre (100 µl) of the substrate was added to each well and incubated in the dark at 21°C for 15 minutes. The reaction was stopped by adding 100µl of stop solution to each well. The plate was then read spectrophotometrically using a biotek ELISA reader at 450 nm. The interpretation of test results was done as follows. For each sample, the competition percentage (S/N %) was calculated as;

$$S/N\ (\%) = \frac{OD\ sample}{OD_{NC}} \times 100\%$$

Interpretation of samples using an S/N (%) was as follows;

1. An S/N less than or equal to 50% are considered positive
2. An S/N greater than 50% and less than or equal to 60 are considered doubtful.
3. An S/N greater than 60% are considered negative.

3.7 Data analysis

Data from the questionnaire was coded and entered into a spreadsheet using Microsoft Excel. Data that was obtained from oral interviews was transcribed and transferred into Microsoft word for further analysis. GIS coordinates were also entered into a spreadsheet using Microsoft Excel to show the study areas. Analysed using the Statistical Package for Social Sciences software (SPSS) version 16. In order to evaluate the potential risk factors associated with PPR disease occurrence, the doubtful samples were considered as positives by taking into consideration the type and nature of disease, its devastating economical effect, similar epidemiological setup of sharing common grazing areas and watering points. The main response variable was whether the animal was positive for PPR antibodies or not. Thereafter, initial descriptive stastics were generated for each of the variables under study. In order to quantify the effects of the potential risk factors on PPR seropositivity were assessed using multivariable logistic regression model. Independent variables that had a p-value of not more than 0.30 in the univariate analysis were included in the model.

The criteria used to determine whether each of the constructed model adequately fitted the data were, a no-significant Hosmer and Lameshow test ($p > 0.05$) and a significant Omnibus test of Model coefficients ($p < 0.05$). All statistical tests were considerd to be significant at $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Descriptive results

4.1.1 Organisation of the District veterinary structure

The study found that not all veterinary camps were manned. Some veterinary camps are too large for one veterinary assistant to manage effectively. For example, Mpulungu district has 11 veterinary camps and only 3 were manned. The staffing levels in the study area is reflected in Table 4.1.

Table 4.1: Staffing levels of veterinary camps in Mbala, Mpulungu, Nakonde and Solwezi (2015)

District	Number of Vet Camps	Number manned	Number unmanned	% manned
Mbala	8	5	3	60
Mpulungu	11	3	8	27
Nakonde	9	4	5	44
Solwezi	5	3	2	60

4.1.2 Source of livelihood

From this study, it was found that 93.5% (29/31) (95% C.I = 84.82 – 102.18%) of farmers depended on crop and livestock farming as a source of living and income generation (Figure 4.1). Most of the farmers (28/31) carried out mixed farming while a single farmer carried out exclusive livestock faming within those that practiced mixed farming

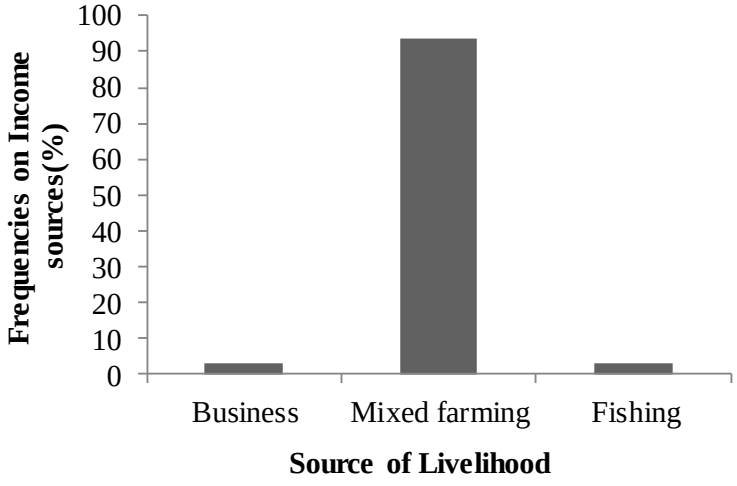

Figure 4.1: Farmers source of livelihood in the study areas

4.1.3 Livestock population

Figure 4.2 presents the various proportion of domestic animals kept by farmers in the study areas. Goats represented a 37 % proportion of the domestic animals kept depicting the most dominant animals kept by the small holder farmers in the four districts. Meanwhile poultry was second to goats (33 %) followed by cattle (24 %), pigs (4%) and sheep (2%).

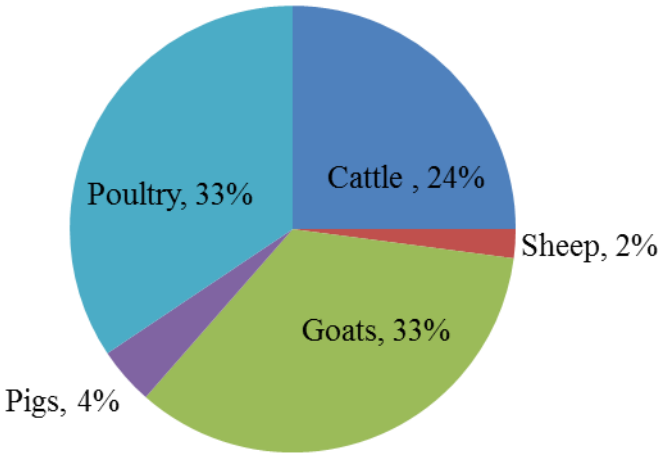

Figure 4.2: Livestock population by types in the study area

4.1.4 Source of livestock for breeding

Table 4.2 shows source of livestock for breeding and the type of market they are sold at in each of the studied districts. Twenty five percent (95% C.I = 9.76 – 40.24%) of farmers bought their livestock from other districts and neighbouring countries and the other 75% (95% C.I = 59.76 – 90.24%) bought the breeding stock within the districts. In Mbala district, 89% of the breeding stock was sourced from within. The study also found that in Mpulungu, 71.4 % (95% C.I = 55.49 – 87.31%) of the source of the breeding stock was sourced from outside the district and Zambia, the rest of the animals were bought from within the Mpulungu. This was similar to Nakonde where 92.8% (95% C.I = 83.7 – 101.9%) of the source of the breeding stock was from within the district and o 3.2% (95% C.I = -3 – 9.4%) of farmers bought from outside Zambia. Combined data for the four district when analysed showed that there is a significant difference ($p<0.001$) in the number of animals coming into the various districts from other districts and countries than within. The study also found that 93 % (95% C.I = 84.3 – 102.6) of small scale farmers sold their livestock in unestablished markets and the rest sold in established markets.

Table 4.2: Source of animals for breeding and selling points

District	Source of breeding stock		Selling Markets	
	Within Districts	Outside Districts	Established	Un-established
Mbala	8	1	0	9
Mpulungu	2	5	0	7
Nakonde	13	1	2	12
Solwezi	0	1	0	1
Total	**23**	**8**	**2**	**29**

4.2 Serology

Table 4.3 shows livestock population, number of animals sampled and cELISA results in the study area. A total of 532 serum samples were analysed to determine the presence of antibodies for PPR virus using cELISA.The analysis revealed that PPR was present in Mbala, Mpulungu, Nakonde and Solwezi. A 1.7 % (9/532) (95% C.I = 0.6 – 2.8%) sero-positivity was found positive for PPR virus antibodies. The district sero-positivity was 0.5 % (95% C.I = -0.47 – 1.47%) for Mbala, with 1.80 % (95% C.I = -0.66 – 4.26%) in Mpulungu, 2.4% (95% C.I = 0.06 – 4.74%) in Nakonde and 4.0 % (95% C.I = -1.43 – 9.43%) for Solwezi . The doubtful were 62 out of 532 samples examined.Among the doubtful, some had similar epidemiological setup of sharing common grazing areas and watering points, while others were of the same herd with the strong positives.

Table 4.3: Livestock population, number of animals sampled and cELISA results in the study area

Province	District	Population		#Sampled	% Completion			%
		Goats	Sheep		(≤50)	(50- 60)	(>60)	Positve
Northern	Mbala	19,420	575	205	1	2	202	0.50
	Mpulungu	7,507	506	112	2	21	89	1.80
Muchinga	Nakonde	15,715	798	165	4	38	123	2.4
North-Western	Solwezi	10,350	854	50	2	1	47	4.0
	Total	**52,992**	**2,733**	**532**	**9**	**62**	**461**	**1.70**

Table 4.4 presents sex distribution of PPR positive from 500 samples. Fifty four (10.8%) of the positive animals were females and 14 (2.8%) were males. There was no statistical difference between the sex as males were equally likely of having PPR disease as females (p =1.000, Odds ratio =1.001). The majority of positive animals were in the range of 25-36 months for both females and male (Figure 4.3). There was no statistical difference among the age groups (p=0.092).

Table 4.4: Sex distribution of PPR positive animals

Sex	Number of animals	Positivity (%)
Female	397	10.8
Male	103	2.8

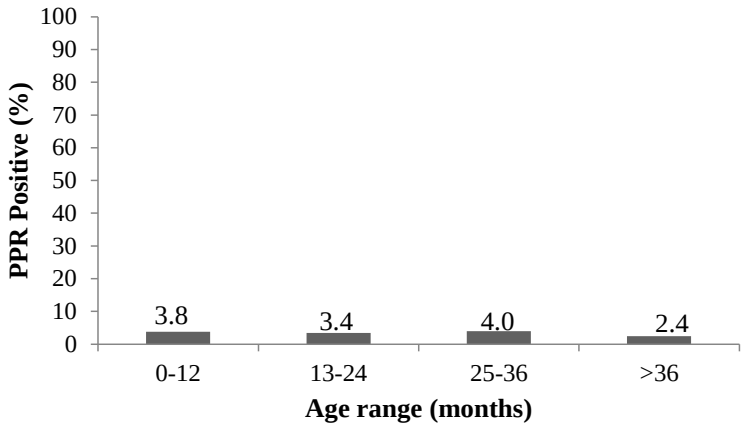

Figure 4.3: Sero-positivity of ppr in the different age groups of 0-12 months (n=19), 13-24months (n=17), 25-36 months (n=20) and >36 months (n=12)

4.3 Risk factors

4.3.1 Veterinary clearance, livestock trading and awareness on PPR

About 65 % (95% C.I = 48.21 – 81.79%) of small holder farmer do not clear their animals with the veterinary department and only 35 % (95% C.I = 18.21 – 51.79%) presented their animals for clearance (Fig 4.4). When analysed by district, the data showed about 85 % (95% C.I = 72.43 – 97.57%) of small holder farmers not having their animals cleared with the veterinary department in Mbala and Mpulungu districts (appendix III). The study also found that at least 57.1 % (95% C.I = 39.68 – 74.52%) of the farmer presented the animals for clearance in Nakonde. The study also found that about 65 % (95% C.I = 48.21 – 81.79%) of the farmers traded in goats within the district and neighbouring countries (appendix III).

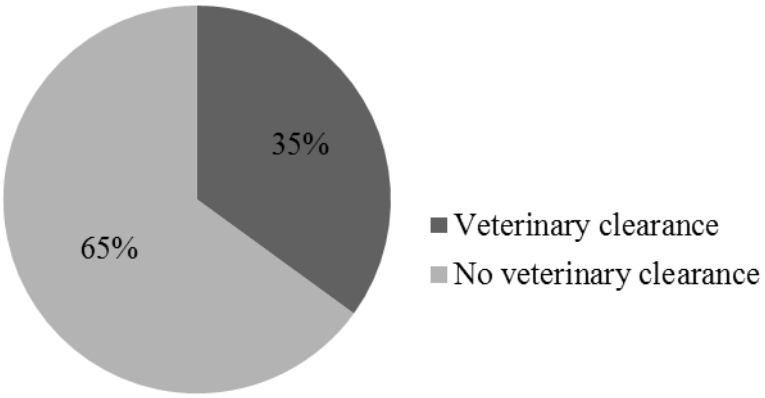

Figure 4.4: Overall proportion of traders seeking veterinary clearance in the study areas

The study revealed that 42 % (95% C.I = 24.63 – 59.37%) of farmers were not aware of PPR disease while 58 % (95% C.I = 40.63 – 75.37%) were aware (Fig 4.5).

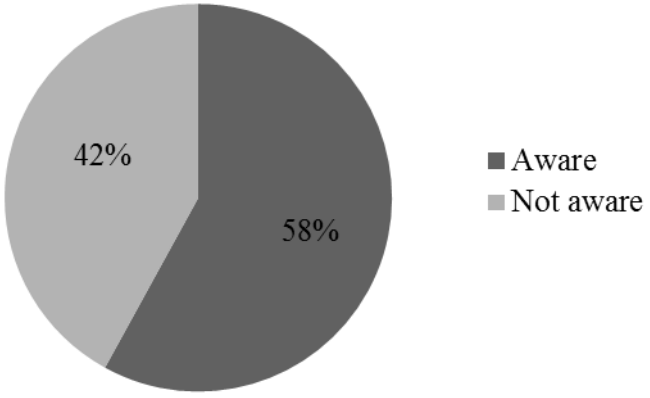

Figure 4.5: Level of PPR awareness among the farmers

4.3.2 Grazing system

The study found 26 % (95% C.I = 10.56 – 41.44%) of the smallholder farmers practiced transhumance type of grazing system. The rest of the farmers 74% (95% C.I = 58.56 – 89.44%) did not move their animals from one place to another in search of grazing land.

4.3.3 Disease reporting

The study found that about 10 % (95% C.I = -0.56 – 20.56%) of farmers in the study reported to the Veterinary department when the animals got sick and 90 % (95% C.I = 79.44 – 100.56%) did not report when their animals got sick (Fig 4.6).

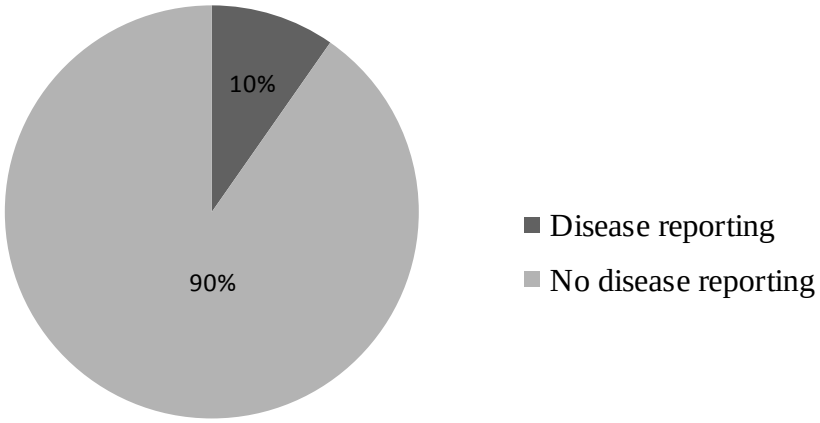

Figure 4.6: Farmers reporting of diseases affecting livestock to Veterinary staff In the study

4.3.4 Regulation on animal movement

The study also observed that only 26% (8/31) (95% C.I = 10.56 – 41.44%) of farmers were aware of the law that is in place on the regulation of animal movement in Zambia while the other 74% (23/31) (95% C.I = 58.56 – 89.44%) were not aware.

4.3.5 Association of risk factors to disease presence

The study found that among the potential risk factors, trade and regulation on animal movement were found to be significantly affecting the presence of PPR ($p<0.001$). It was also observed that those involved in trading were 10.996 times likely to have disease than those who were not trading with other districts and neighbouring countries. There was no significance difference in awareness, veterinary clearance, disease reporting and type of grazing ($p=0.298$, 0.062, 0.099 and 0.110) respectively.

Table 4.5: Risk factors evaluated for the association with the occurrence of PPR in the study areas

Factor	Response	Number of samples	P-value	Odds Ratio	95 % CI
PPR awareness	No	223	0.294	0.738	0.437-1.248
	Yes	227			
Veterinary clearance	No	353	0.062	0.584	0.344-0.992
	Yes	147			
Trade	Outside	252	0.000	10.996	4.917-24’590
	Within	248			
Disease reporting	No	458	0.099	3.367	0.795-14.268
	Yes	42			
Grazing system	Local	359	0.110	0.631	0.369-1.080
	Transhumance	141			
Regulation on movement	No	379	0.000	0.314	0.185-0.534
	Yes	121			

The multivariate analysis, using logistic regression,with confidence interval of 95%, was used to assess the association between the identified significant risk factors in the univariate analysis in combination with a positive cELISA status for PPR. A multivariable logistic regression was performed to ascertain the effects of age, veterinary clearance, PPR awareness, trade, grazing system, disease reporting and awareness on animal movement regulation on the likelihood of having PPR disease and the results were as shown in table 4.6 below. Those who were not aware were 3.194 times more likely to have PPR disease than those who were.aware.Those who were trading between districts were 12.995 times more likely of having PPR. From the results, there was statistical significance of PPR disease presence with risk factors such as PPR awareness($p=0.006$), trade ($p<0.001$), disease reporting ($p<0.001$), and awareness on animal regulation ($p<0.001$) respectively.

Table 4.6: Risk factors associated with PPR disease presence using a multivariable logistic regression model

Risk Factors	Odds Ratio	Std Err.	P-value	95.0% C.I. for Odds Ratio	
				Lower	Upper
PPR awareness(No/Yes)	3.194	0.422	0.006	1.396	7.309
Veterinary clearance(No/Yes)	0.611	0.331	0.137	0.320	1.169
Trade(Yes/No)	12.995	0.428	0.000	5.611	30.093
Disease reporting(No/Yes)	21.410	0.804	0.000	4.427	103.547
Grazing system(No/Yes)	1.151	0.368	0.701	0.560	2.366
Awareness on movement regulation(No/Yes)	0.171	0.448	0.000	0.071	0.411
sex(F/M)	0.618	0.382	0.208	0.292	1.307
Age range of animals	1.396	0.145	0.022	1.050	1.856

Number of Observations in the model = 31

Hosmer and Lemeshow Chi-square (8) =7.190, Probability>Chi-square=0.516

4.4 Questionnaire response from the Veterinary staff

Fifty percent (50%) (3/6) of the Veterinary staff were aware of PPR and the disease presence in Tanzania and Democratic Republic of Congo. The study also found that from the three staff, only two were able to diagnose PPR by clinical signs. The study also found that veterinary staff were aware of the sheep and goats moving between Zambia and the affected neighbouring countries and that the direction of movement was in either direction. Further the study noticed that goats are the ones traded more compared to sheep. Among the reasons that were speculated to drive the cross border trade in goats were due to high demand for meat and inter-marriages. The movements of the animals were most often not accompanied by stock movement permits.

Farmers in Mpulungu district used Isoko camp to bring animals from Tanzania into Zambia and also use Lake Tanganyika and unknown routes on the borders with Tanzania. In Nakonde, traders used Ndingindi road in Tanzania enroute to Kasama, Mbala road enroute to Kasama and Uzinji road enroute into Zambia. In Mbala, traders use Mbala road and illegal routes to bring animals from Tanzania. It was also found that the common diseases which the famers faced included heart water, endoparasites, pneumonia and mange. Other challenges was the handling of reports for the sick animals through the District Veterinary Offices (DVOs) if any of the farmers was affected. Thereafter, the affected farmers are visited to investigate what the problem is and if there animals are dead, postmortems are carried out. The cases are followed up until the affected animals recover.

CHAPTER FIVE

5.0 DISCUSSION

Peste des petits ruminants (PPR) disease causes varying degree of morbidity and mortality in susceptible population (Radostits *et al.*, 2000). PPR is a disease of increasing importance in Africa, particularly in areas where small ruminants form an important component of agricultural food production such as Zambia. In the SADC region, Angola, DR Congo and Tanzania have been affected (Swai *et al.*, 2009, FAO 2010, Muse *et al.*, 2012) putting Zambia at a risk of getting the disease.

Prior findings by Chazya *et al.* (2014) established that there was a high risk of occurrence of PPR in Northern province of Zambia through the importation of live goats from Tanzania. Therefore, it was against this background that this study was carried out with the main objective of evaluating the epidemiological status of PPR in small ruminants in Muchinga, Northern and North-Western provinces of Zambia. The specific objectives were to determine the seropresence of PPR in areas bordering Tanzania and Democratic Republic of Congo (DRC) and to assess the levels of PPR awareness and risk factors associated with PPR among small ruminant farmers.

The districts of Mbala, Mpulungu, Nakonde and Solwezi were found positive for PPRV antibodies. The sero-positivity of PPR in the studied districts of Mbala, Mpulungu, Nakonde and Solwezi were (0.5%, 1.8%, 2.4% and 4.0%) respectively. Thus these districts were considered to be high risk areas for PPR occurrence due to their proximity with PPR affected DR Congo and Tanzania. This finding confirms Chazya et al. (2014) results who found PPR likelihood of occurrence to be higher in the three districts except for Solwezi. The sero-positivity found in this study is much lower compared to Zambia's neighbouring countries. For example, Swai *et al*. (2009) found a PPR seroprevalence of 39.8 % in sheep and 49.5 % in goats in Tanzania. The age groups sero-positivity were significantly different ($p = 0.022$) in all the districts. This is contrary to Shuaib *et al.* (2014) who found that the seroprevalence among age groups not to be significantly different in Sudan.

From this study, it was found that goats were reared more compared to sheep. This demonstrates that goats also contributes a significant proportion to the socioeconomic status of the smallholder farmers in the studied areas. This therefore, justifies the need to monitor diseases such are PPR which can causes a higher mortality rate in the affected population. The possible reasons why goats are preferred among other livestock because they are prolific, drought resilient and cheaper to obtain (Ngategize, 1989; Jones and Thornton, 2009).

The study found that there was no significance difference in disease presence among farmers who did not obtain veterinary clearance and those that obtained. The regulation of animal movement was found to significantly affect the presence of PPR. This is similar as in West Africa and where PPR spread from Ivory Coast to other Western African countries and SADC region (Gargadennec and Lalanne 1942; Kivaria *et al.,* 2009; Swai *et al.,* 2009; FAO 2013). The higher level of non-clearance of animals presents a great hazard to the country's livestock industry with PPR diseases. These findings shows that there is illegal movement of animals as many people move animals without screening leading to high chances of diseases such as PPR occuring. This result is consistent with Chazya *et al.* (2014) who found the risk of PPR being introduced into Zambia through Mbala, Mpulungu and Nakonde Districts to be high.

In this study, it was found that there was statistical evidence that trade animal movement was found to be significantly affecting the presence of PPR. It was also observed that those were 10.996 times likelhood of having the disease as a result of trading in small ruminnts. This is expected similar to Roeder *et al.* (1999) who has documented the PPR virus to be transmitted when animals sneeze or cough, since the virus is found in the discharges from the eyes, nose and mouth of infected animals. Therefore, the contact and movement of animals from the affected to the unaffected areas play a significant role in the transmission of PPR disease especially where communal grazing type of system is practiced. In Tanzania, PPR was first reported in 2008 in the Northern zone districts bordering Kenya (Karimuribo *et al.,* 2011). Later the disease was introduced into Southern Tanzania in 2009 through goats that were purchased from a livestock market on the outskirts of Dar es Salaam city (Muse *et al.,* 2012). Therefore, there is need to have supervised markets where farmers sell their animals for easy control of PPR as animals mingle during the period of marketing the disease is transmitted. Therefore, the disease spreads to other farms through addition of breeding stock from infected goats and sheep from the market.

The study found that there was a significance difference in disease presence among farmers who were aware of PPR disease and those who were not aware. The veterinary services rely on stockowners or on veterinarians attending to livestock to notify the possible occurrence of an exotic disease. Notification need to be prompt and accurate so that all outbreaks are identified as soon as possible without raising too many false alarms. Prompt and accurate notification can only be achieved if the veterinary profession and stockowners are aware of the danger posed by PPR and are conversant with the clinical signs of the disease. For this purpose, countries affected should ensure that awareness of the disease is maintained within

the veterinary profession, in the livestock/agricultural community and by the general public (SADC 2012a). Though it may not sound very practical, continuous education and awareness creation using a combination of tools/techniques at household/ farm level might important to help educate the farmers on diseases affecting their animals (SADC 2012a).

The study found that some of the Veterinary staff did not know what PPR was and how it is diagnosed. It was also observed that animals move between countries without stock movement permit and this leads to PPR spreading from affected areas to clean areas. The study also observed that the staffing levels in the study areas also affected Veterinary extension services in that most of the camps did not have Veterinary staff. Despite other camps having staff, the coverage areas were big and lack of transport to use in offering extension services worsed the situation. The lack of staff to offer extension services also contributed to farmers moving moving the animals illegally showing that most of the animal move informally. This was in agreement to Wakhusama *et al.* (2011) who observed most of the crossborder movement of goats and sheep in the high risk boarders of Zambia to be informal. Therefore, there is need of strengthening the veterinary services along the country borderings on both sides by intensifying surveillance, monitoring, and livestock movement controls. Lack of proper veterinary services and adequate infrastructure may facilitate disease transmission.

To enable proper and sustainable control of PPR is a challenging task. During this study some of the challenges that were faced were; farmers refusal to have the animals sampled. This was because many researchers have been collecting samples without giving feedback to the farmers. Moreover, most of the veterinary camps did not have Veterinary staff leading to some of the areas not sampled. The lack of transport despite some of the camps being manned by veterinary staff in place, therefore, it was difficult to mobilise farmers due to lack of transport. The limitation of the study was that the resources were not enough, leading to some of the areas not sampled.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

Conclusion

PPR is one of the most important economical diseases affecting the small livestock production and affecting trade. The present study has confirmed the presence of PPR antibodies in goats and sheep and this confirmed natural transmission in these animals without clinical disease in Mbala, Mpulungu, Nakonde and Solwezi. The apparent absence of overt clinical presentation in these animals may have been due to failure to diagnose the disease by the farmers and veterinary staff. Among the risk factors that were evaluated to determine PPR occurrence in the study area, trade was found to significantly affect the presence of PPR. It was also observed that the disease was more likely to occur among farmers who lacked veterinary clearance, failure to report diseased animals and those who were not aware of the disease

Recommendations

Based on the observations and results obtained in this study, the following are the recommendations.

1. There is need to determine the extent of PPR in other districts bordering Tanzania, DR Congo and Angola.
2. Assess the extent to which PPR could have spread to other parts of Zambia
3. Confine the disease through initiation of surveillance networks, focused 'ring' vaccination and animal movement restriction to other parts of the country.
4. Farmers and Veterinary staff in the high risk areas need to be educated on PPR.
5. There was need to strengthen veterinary extension services through deployment of Veterinary staff in camps that did not have staff.

REFERENCES

1. Abraham, G. and Berhan, A. (2001). The use of antigen-capture enzyme-linked immunosorbent assay (ELISA) for the diagnosis of rinderpest and peste des petits ruminants in Ethiopia. Tropical animal health and production 33(5), 423-30.

2. Abubakar, M., Jamal, S.M., Hussain, M. and Ali, Q. (2008). Incidence of peste des petits ruminants (PPR) virus in sheep and goat as detected by immuno-capture ELISA (IcELISA). Small ruminants Research 75, 256-259.

3. Abu Elzein, E.M.E., Hassanien, M.M., Alfaleg, A.I.A, Abd Elhadi, M.A. and Housawi, F.M.T. (1990). Isolation of PPR virus from goats in Saudi Arabia. Vet. Rec., 127: 309-310.

4. Abu Elzein, E.M.E., Housawi, F.M.T., Bashareek, Y., Gameel, A.A., Al- Afaleq, A.I. and Anderson, E.C. (2004). Severe PPR infection in Gazelles kept under semi-free range conditions in Saudi Arabia. J. Vet. Microbiology. B 51 (2), 68-71

5. Albina, E., Kwiatek,O., Minet, C., Lancelot, R., Servan, R de Almeida. and Libeau, G. (2013). Peste des petits ruminants, the next eradicated animal disease, Vet. Microbiology. 165:38-44.

6. Anderson, E. C., Hassan, A., Barrett, T. and Anderson, J. (1990). Observation on the pathogenicity for sheep and goats and the transmissibility of the strain of virus isolated during the Rinderpest outbreak in Sri Lanka in 1987 . Vet. Microbiology 21: 309-318.

7. Anderson, J and J. A. McKay, J. A.(1994) The Detection of Antibodies against Peste des Petits Ruminants Virus in Cattle, Sheep and Goats and the Possible Implications to Rinderpest Control Programmes. Epidemiology and Infection. 112(1):225-231

8. Aslam, M., Abubakar, M., Anjum, R., Saleha, S. & Ali, Q. (2009). Prevalence of Peste Des Petits Ruminants Virus (PPRV) in Mardan, Hangu and Kohat District

of Pakistan; Comparative Analysis of PPRV Suspected serum samples using Competitive ELISA (cELISA) and Agar Gel Immunodiffusion (AGID). Veterinary world 2(3), 89-92.

9. 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: 119-124.

10. Balamurugan, V., Hemadri, D., Gajendragad, M. R., Singh, R. K. and Rahman, H. (2013). Diagnosis and control of peste des petits ruminants: a comprehensive review. PMCID: PMC3889233.doi: 10.1007/s13337-013-0188-2

11. Banyard, A.C., Baron, M.D. and Barrett, T. (2005). A role for virus promoters in determining the pathogenesis of rinderpest virus in cattle. Journal of General Virology 86: 1083-92

12. Banyard, A.C., Parida, S., Batten, C., Oura, C., Kwiatek, O. and Libeau, G. (2010). Global distribution of peste des petits ruminants virus and prospects for improved diagnosis and control. Journal of general virology 91(Pt 12), 2885-97.

13. Barrett, T., Subbarao, S.M., Belsham, G.J. and B.W.J. Mahy. (1991) The molecular biology of the morbilliviruses, pp. 83-102. In D.W. Kingsbury (ed.) The paramyxoviruses. Plenum Press. New York.

14. Barrett, T., Visser, I.K.G., Mamaev, L., Goatley, L., Bressem, M.F. and Van Osterhaus, A.D.M. (1993a). Dolphine and porpoise morbilliviruses are genetically distinct from phocine distemper virus. Virology, 193: 1010-1012.

15. Barrett, T., (2001) Morbilliviruses: dangers old and new. In: Smith, G.L., Mc Cauley, J.W., Rowlands, D.J. (Eds.), New Challenges to Health: The Threat of Virus Infection: Society for General Microbiology, Symposium 60. Cambridge University Press, pp. 155-178.

16. Barrett, T., Banyard, A.C. and Diallo, A. (2005). Molecular biology of the morbilliviruses. In: Barrett T, Pastoret P-P, TaylorWP, editors. Rinderpest and

peste des petits ruminants. Virus plagues of large and small ruminants. Amsterdam: Academic Press, Elsevier; p. 31-67.

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

18. Couacy-Hymann, E., Bidjeh, K., Angba, A., Domenech, J., and Diallo, A. (1995). Protection of goats against RP by vaccination with attenuated PPR virus. Res. Vet.Sc.59: 106-109.

19. 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: 17-25

20. Chazya, R., Muma, J.B., Mwacalimba, K.K., Karimuribo, E., Mkandawire, E and Simuunza M (2014). A Qualitative Assessment of the Risk of Introducing Peste des Petits Ruminants into Northern Zambia from Tanzania, Hindawi Publishing Corporation, Veterinary Medicine International Volume 2014, Article ID 202618, 10 pages

21. Choi, K.S., Nah, J.J., Ko, Y.J., Kang, S.Y. and Jo, N.I. (2005). Rapid competitive enzyme-linked immunosorbent assay for detection of antibodies to Peste des petits ruminants virus. Clinical and Diagnostic Laboratory and Immunology, 12(4): 542-547. Diallo, A. (1988). Peste bovine et peste des petits ruminants: des menaces constantes contre l'élevage dans beacoup de pays en development. Impact : Science et Société 150: 191-204.

22. Diallo, A., Barret, T., Barbon, M., Shaila, M., Subbarao, T. and William, P. (1989a). Differentiation of rinderpest and peste des petits ruminantss viruses using specific cDNA clones. J. Virol. Methods. 23: 127-136.

23. Diallo, A., Minet, C. and Goff, C. (2007). The threat of peste des petits ruminants: progress in vaccine development for disease control. In Vaccine; 4th International Veterinary Vaccines and Diagnostic Conference, Oslo, Norway, 25-29 June 2006. 2007; 25(30)5591-5597.

24. 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), 711-7.

25. Domingo, M., Ferrer, L., Pumarola, M., Marco, A., Plana, J., Kennedy, S., M. McAliskey, M. and Rima, B. K. (1990). Morbillivirus in dolphins. Nature 348:21.

26. El Hag, B. and Taylor, T. W. (1984). Isolation of PPR virus from Sudan. Res Vet Sci. 36:1–4. [PubMed]

27. 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, (2010), Volume 58. No. 3.185-193.

28. EMPRES; Emergency Prevention System (2009). Peste des petits ruminants (PPR): an increasing threat to small ruminant production in Africa and Asia. FAO: 1-8.

29. FAO (2010). Deadly animal virus Peste des Petits Ruminants threatens to spread to Southern Africa, *Goal Geology*, viewed 16 January 2011, from -animal-virus-peste-des-petits ruminants-threatens-to-spread-to-southern-africa/8302

30. Food and Agriculture Organization of the United Nations (FAO) (2011).World Livestock Livestock in food security. Rome, Italy.

31. FAO, (2013a). Livestock Epidemic Causing Havoc in Democratic Republic of the Congo, vol. 1, FAO, Rome, Italy, 2013.

32. Food and Agriculture Organization of the United Nations (FAO) (2013). World livestock 2013: changing disease landscapes. FAO, Rome, 130 pp. Available at: www.fao.org/ docrep/019/i3440e/i3440e.pdf (accessed on 3 December 2014).

33. Gargadennec, L. and A. Lalanne. 1942. Peste des petits ruminants. Bull Services Zootechn Epizoot Afric Occident (French with English abstract) 5: 16-21.

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

35. Government of the Republic of Zambia,(2006). Fifth National Development Plan 2006-2010, Ministry of Finance and Planning, Lusaka. Zambia.

36. Hamdy, F.M., Dardiri, A.H., Nduaka, O., Breese, S.R., and Ihemelandu, E.C. (1976). Etiology of the stomatitis pneumocenteritis complex in Nigerian dwarf goats. Can. J. Comp. Med. 40: 276-284.

37. Jones, P. G., Thornton, P. K., 2009. Croppers to livestock keepers: livelihood ransitions to 2050 in Africa due to climate change. Enviroment Science and Policy; 12:427-437.

38. Karimuribo, E. D., Loomu, P. M. L. and Mellau, S. B. (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, vol. 1, no. 3, pp. 184–187.

39. Khalafalla, A.I., Saeed, I.K., Ali, Y.H., Abdurrahman, M.B., Kwiatek, O. and Libeau, G. (2010). An outbreak of peste des petits ruminants (PPR) in camels in theSudan,ActaTropica116,161165.http://dx.doi.org/10.1016/j.actatropica.2010.08.002, PMid: 20707980

40. Khan, H.A., Siddique, M., Sajjad ur Rahman, Abubakar, M. and Ashraf, M. (2008), The detection of antibody against peste des petits ruminants virus in sheep, goats, cattle and buffalo, *Tropical Animal Health and Production* 40, 521–527. http://dx.doi.org/10.1007/s11250-008-9129-2, PMid:18716909

41. Kinne, J., Kreutzer, R.., Kreutzer, M.., Wernery, U. and Wohlsein, P. (2010. Peste des petits ruminants in Arabian wildlife. Epidemiol. Infect. 138 (8), 1211-1214.

42. Kitching, R.P. (1988). The economic significance and control of small ruminant viruses in North Africa and West Asia. In increasing small ruminant productivity in semi-arid areas.

43. 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, paper presented at the 27th Tanzania Veterinary Association Scientific Conference, Arusha, Tanzania, 04-06 December.

44. Kivaria, F.M., Kwiatek, O., Kapaga, A.O., Swai, E.S., Libeau, G. and Moshy, W. (2013). The incursion, persistence and spread of peste des petits ruminants in Tanzania: Epidemiological patterns and predictions, *Onderstepoort Journal of Veterinary Research* 80(1), Art #593, 10 pages. http:// dx.doi.org/10.4102/ojvr. v80i1.593

45. Kusiluka, L. and Kambarage, D. (1996).Small stocking development. Diseases of Small Ruminants. A Handbook: Common Diseases of Sheep and Goats in Sub-Saharan Africa. Diseases caused by viruses. Available via LPP/DFID. www.smallstock.info/research/reports/R5499/content.htm pp.66 Accessed 15 Jan 2009

46. Kwiatek, O., Ali,Y. H., Saeed, I. K., Khalafalla, A. I., Mohamed, O.I., Obeida, A.A., Abdelrahman, M. B., Osman, H. M., Taha, K. M., Abbas, Z., El Harrak, M., Lhor, Y.,Diallo, A., Lancelot, R., Albina, E. and Libeau. G. (2011). Asian lineage of peste des petits ruminants virus, Africa. Emerg. Infect. Dis. 17:1223-1231

47. Lefèvre, P.C. and Diallo, A. (1990). Peste des petites ruminants. Revue Scientifique Office International des Epizooties 9: 951-965.

48. Lefevre P.C., Diallo A., Schenkel F., Hussein S. & Staak G. (1991). Serological evidence of peste des petits ruminants in Jordan. The Veterinary Record 128, 110.

49. Lefèvre, P.C., Blancou J., Chermette R. and Uilenberg G. (2010). Infectious and Parasitic Diseases of Livestock, Ed TEC et DOC, Lavoisier, Paris, France, 225-244.

50. 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), 50-5.

51. McCullough, S. J., McNeilly, F., Allan, G. M., Kennedy, S., Smyth, J. A., Cosby, S. L., McQuaid, S. and Rima, B. K. (1991). Isolation and characterisation of a porpoise morbillivirus. Arch. Virol. 118: 247-252.

52. Ministry of Agriculture and Livestock (2012). The Livestock sector in Zambia-For economic growth and poverty reduction.

53. Munir, M. (2011). Diagnosis of Peste des Petits Ruminants under limited resource setting: A cost effective strategy for developing countries where PPRV is endemic. 1st. ed: VDM Verlag Dr. Müller

54. Murphy, S.K. and Parks, G.D. (1999). RNA replication for the paramyxovirus simian virus 5 requires an internal repeated (CGNNNN) sequence motif. Journal of Virology 73: 805-9.

55. Muse, E. A., Matondo, R. B., Karimuribo, E. D., Misinzo, G., Albano, M. O. and Gitao G. C.(2011). Clinico- pathological findings of the outbreak of Peste des Petits Ruminants (PPR) in Tandahimba district, southern Tanzania. Research Opinions in Animal and Veterinary Sciences.. 2:256-26

56. Muse, E.A., Karimuribo, E.D., Gitao, G.C., Misinzo, G., Mellau, L.S.B. and Msoffe, P.L.M. (2012). Epidemiological investigation into the introduction and factors for spread of Peste des Petits Ruminants, southern Tanzania, Onderstepoort Journal of Veterinary Research 79(2), Art. #457, 6 pages. http://dx.doi. org/10.4102/ojvr.v79i2.457

57. Norrby, E. and Oxman, M. N. (1990). Measles virus, PP; 1013-1044. In B.N. Fields et al., Virology, 2nd edition, Vol. I, Raven Press, New York 1: 1013-1044.

58. Nussieba, A.O., Ali, A.S., Mahasin, E.A.R. and Fadol, M.A. (2009). Antibody seroprevalences against peste des petits ruminants (PPR) virus in sheep and goats in Sudan, Tropical Animal Health and Production 41, 1449–1453. http://dx.doi.org/10.1007/s11250- 009-9333-8, PMid:19798772

59. Ngategize, P. K.(1989). Constraint identification and analysis in African Small ruminant systems. In: R. T. Wilson, A. Wilson and M. Zebu (eds.), African Small Ruminant Research and Development, ILCA, (Addis Ababa, Ethiopia), 7-22.

60. Njagi, L. (2009). Presentation during the TCP/RAF/3113(E) Closure Workshop Entebbe, Uganda on Peste des Petits Ruminants (PPR) Management and Control; Kenya case Presentation during the TCP/RAF/3113(E) Closure Workshop Entebbe, Uganda on Peste des Petits Ruminants (PPR) Management and Control Kenya case, Entebbe, Uganda.

61. OIE, (2010). Manual of the Diagnostic Tests and Vaccines for Terrestrial Animals, vol. 1, Office International Des Epizooties, Paris, France, 5th edition.

62. OIE. (2012). Peste des petits ruminants. Chapter 4: Disease factsheets: 263-267

63. Ozkul, A., Akca, Y., Alkan, F., Barrett, T., Karaoglu, T., Dagalp, S.B., Anderson, J., Yesilbag, K., Cokcaliskan, C., Gencay, A. and Burgu, I. (2002). Prevalence, distribution and host range of peste des petits ruminants virus, Turkey. Emerg. Infect. Dis. 8, 708-712.

64. Pestana, E. A., Belak. S., Diallo, A., Crowther, J. R. and Viljoen, G. J. (2010). Early, Rapid and Sensitive Veterinary Molecular Diagnostics-Real Time PCR Applications, DOI 10.1007/978-90-481-3132-7_5.

65. Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchcliff, K.W. (2000). Peste des petits ruminants. In: Veterinary Medicine, 9th edition .W.B. Saunders, London. 1077-1079.

66. Radostits O.M., Gay C.C., Hinchcliff K.W. and Constable P.D. (2007). Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats, 10th Ed. Saunders Elsevier, London, 1242–1244. 19.

67. Raghavendra, L., Setty, D.R.L. and Raghavan, R. (2000). Clinico-biochemical changes in sheep and goats experimentally infected with Vero cell adapted peste-des petits ruminants (PPR) virus. International Journal of Animal Science, 15 (2): 171-173.

68. Roeder, P.L., Abraham, G., Kenfe, and Barrett, T. (1994). PPR in Ethiopian goats. Tropical. Animal Health. Production, 26: 69-73.

69. Roeder, P.L. and Obi, T.U. (1999). Recognizing peste des petites ruminants: A field manual, FAO Animal Health Manual (5), 28 pp.

70. Roger, F., Gebre, Y. M., Libeau, G., Diallo, A., Yigezu, L.M. and Yilma, T. (2001). Detection of antibodies of rinderpest and peste des petits ruminants viruses(Paramyxoviridae, Morbillivirus) during a new epizootic disease in Ethiopian camels (Camelus dromadarius). Rev. Méd. Vét., 152: 265-268.

71. SADC. (2012a). SADC Pestes des petits ruminants (PPR) control and eradication working group Gaborone, Botswana, SADC: 1-4.

72. SADC. (2012b). SADC Control Strategy for Pest de petits ruminants. Gaborone, Southern African Developmenet Community: 1-24.

73. SADC . 2012. Status of Animal Health Bulletin 2.

74. Saliki, J., Libeau, G., House, J.A., Mebus, A. and Dubov, E.J. (1993). Monoclonal antibody based blocking ELISA for specific detection and titration of PPR virus antibody in caprine and ovine sera. Journal of Clinical Microbiology 31: 1075 - 1082.

75. Shaila, M.S., David S., Foryth, M.A., Diallo, A., Goatley, L., Kitching, R.P. and Barret, T. (1996). Geographical distribution and epidemiology of PPR viruses. Virus Research 43: 149-153.

76. Shankar, H., Gupta, V.K. and Singh, N., (1998). Occurrence of pest des petitis ruminants like disease in small ruminants in Uttar Pradesh. Indian J. Anim. Sci., 68: 38-40.

77. Shuaib, YA., El-Fadil, A.A. M., Abdel-Rahman,M. E., Negussie, H. and Zessin.,K.H. (2014). Seroprevalence and risk factors of Peste des petits ruminants in sheep in the Kassala and North Kordofan States of the Sudan. Inter J Vet Sci, 3(1): 18-28. www.ijvets.com

78. Sitko, N., Chapoto, A., Kabwe, S., Tembo, S., Hichaambwa, M., Lubinda, R., Chiwawa, H., Mataa, M., Heck, S. and Nthani D. (2011). Technical Compendium: Descriptive Agricultural Statistics and Analysis for Zambia in Support of the USAID Mission's Feed the Future Strategic Review, Working paper No.52.Food Security Research Project, Lusaka, Zambia.

79. Steinfeld, H., Wassenaar, T. and Jutzi, S. (2006). Livestock production systems in developing countries: status, drivers, trends. In Animal production food safety challenges in global markets (S.A. Slorach, ed.). Rev. sci. tech. Off. int. Epiz., 25 (2), 505-516.

80. Srinivas, R.P. and Gopal, T. (1996). PPR: A new menace of sheep and goats. Livestock adviser

81. Sumberg, J.E. and Mack, S.D. (1985). Village production of dwarf goats and sheep in Nigeria. Tropical Animal Health Production, 17(3), 135-140

82. 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, vol. 33, no. 8, pp. 927-936.

83. Taylor, W.P. (1984). The distribution and epidemiology of peste des petits ruminants. Prev. Vet. Med., 2, 157-166

84. Taylor, W. P. (1979). Serological studies with the virus of peste des petits ruminants in Nigeria. Res. Vet. Sc. 26: 236-242.

85. Thrusfield, M. (1997). Veterinary Epidemiology 2nd Edition Blackwell Science Ltd. Pages 187-192.

86. Wakhusama, S., Sinkala, Y. and Chisembele, C.C. (2011). Rapid assessment on peste des petits ruminants situation in Zambia, VACNADA, AU-IBAR: 1-16.

87. Wambura, P. N. 2000. Serological evidence of the absence of peste des petits ruminants in Tanzania. Veterinary Record 146(16): 473-4.

88. World Organisation for Animal Health (2008). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. OIE, Paris.

89. World Organization for Animal Health and FAO (2015) .Global control and eradication of peste des petits ruminants; Investing in veterinary systems, food security and poverty alleviation.

APPENDICES

Appendix I: Questionnaire

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VETERINARY MEDICINE

DEPARTMENT OF DISEASE CONTROL

.

Epidemiology of Peste des Petits Ruminants (PPR) in the high risk areas of Northern, Muchinga and North-Western Provinces of Zambia

Introduction

This research project is focusing on determining the presence of Peste des Petits ruminants (goat plague) in the selected districts identified to be at a high risk of having the disease in Northern and North-western Provinces of Zambia. In this survey, you will be asked to complete a short questionnaire. This questionnaire aims to collect information on PPR and will be used to enhance and supplement disease control efforts. The assurance is that all the information you will provide shall be confidential and will only be used for the purpose of this study. The research will abide by ethical codes. Samples will be collected, screened for this disease and feedback shall be given. I would like to thank you for taking your time to participate in this study.

PART 1

Name of the Interviewer: ----------

Province: ----------

District: ----------

Veterinary Camp: ----------

Village/Farm: ----------

Geographical Coordinates

Latitude: ----------

Longitude: ----------

Name of Respondent: ----------

Date of Interview: ----------

PART 2: SOCIAL DEMOGRAPHIC INFORMATION OF INFORMANTS

Thank you for agreeing to participate in this study. I would like to assure you that all the information you will provide shall be treated as confidential and will only be used for the purpose of this study.

1. Respondent ID ----------Village----------

1. Sex of respondent Male/Female

2. Age of respondent (if less than 18 years old end the interview and thank the respondent for his or her time) ----------years

3. What is your main source of livelihood? ----------

Kindly respond to the questions in the appropriate section

Section A- Farmers and Livestock Traders

1. What type of livestock do you own? (Tick)

Cattle/ Sheep/ Goats/ Pigs/ Poultry/ Others

2. Quantify your livestock

Cattle ☐ Sheep ☐ Goats ☐ Pigs ☐ Poultry ☐ Others----------

3. Where do you usually get your livestock for breeding/sale? ----------

4. How many goats and sheep have you bought in the last year and from where? ------

---

5. Do you buy any animals from the neighboring countries? Yes /No

6. If yes, state the countries----------

7. Do you get any Veterinary clearance before you move your livestock? Yes /No

8. Are you aware of any other farmers/traders who buy Goats & Sheep from the neighboring countries? ----------

9. Do you sell goats to well established market? Yes/ No

10. Do you move animals from one area to another in any part of the year in search of pasture or better markets? Yes/ No.

11. If yes in (8) are you aware of any livestock regulations related to animal movements? Yes/No

12. Have you ever heard of the disease Peste des petits ruminants (PPR)? Yes/ No

13. Are you aware of its presence in the neighboring countries? Yes/ No

14. Are you familiar with the clinical manifestation of the disease to the extent that you can diagnose it in case of an outbreak? Yes /No

15. If yes in (12), what are some of the clinical signs of the disease? Please specify----------

---

Section B- Veterinary Staff

1. Respondent ID----------District/Camp----------

2. How long have you worked in the Veterinary field? ----------

3. Are you aware of goat and sheep movements between Zambia and other countries? Yes/ No

4. If Yes in (3), what is the direction of the movements? ----------

---

5. Have the movements been accompanied by stock movement permits? Yes/ No

6. If no to question 5, what could be the estimates of movement in the last one year? ----------

---

7. What do you think could be the reasons driving these cross border movements? ----------

---

8. Are you aware of the routes used by farmers/traders to move the sheep and goats? Give details ----------

9. Have you ever heard of the disease Peste des petits ruminants (PPR)? Yes /No

10. Are you aware of its presence in the Tanzania/ D.R Congo? Yes /No

11. Are you familiar with the clinical manifestation of the disease to the extent that you can diagnose it in case of an outbreak? Yes No

12. If yes in (8), what are some of the clinical signs of the disease? Please specify----------

---

---

13. What is the goat and sheep population in your catchment area? ----------

14. What are the common diseases affecting goats and sheep in your area? ----------

---

15. How do you handle reports of goat/sheep diseases? ----------

---

16. Do you have the capacity to conduct PPR surveillance in your area? Yes No

17. What things do you think would help to improve on your surveillance system?

THANK YOU FOR ANSWERING THE QUESTIONS

Appendix II: Contents of the PPR c-ELISA kit

Sr.	No. Item	Storage
1	Microplates coated with PPR recombinant nucleoprotein	+2°C to +26°C
2	Anti-NP-HRP concentrated conjugate (10X)	5°C(±3°C)
3	Positive Control	5°C(±3°C)
4	Negative Control	5°C(±3°C)
5	Dilution buffer 13	+2°C to +26°C
6	Dilution buffer 4	+2°C to +26°C
7	Wash concentrate (20X)	+2°C to +26°C
8	Substrate solution	5°C(±3°C)
9	Stop solution	+2°C to +26°C

Appendix III:Responses of farmers on PPR awareness, veterinary clearance and trade

	Awareness on PPR		**Veterinary clearance**		**Trade**	
	Yes	No	Yes	No	Yes	No
Mbala	5	4	1	8	0	9
Mpulungu	1	6	1	6	5	2
Nakonde	12	2	8	6	14	0
Solwezi	0	1	1	0	1	0
Total	**18**	**13**	**11**	**20**	**20**	**11**

Appendix IV: GPS coordinates for the farms sampled

Province	District	Veterinary Camp	Latitude	Longitude	Respondent Id	Village
Northern	Mbala	Mbala Central	08.71027°	031.50193°	MB01	Chizombwe
Northern	Mbala	Mbala Central	08.70299°	031.49575°	MB02	Chizombwe
Northern	Mbala	Mbala Central	08.70292°	031.49536	MB03	Chizombwe
Northern	Mbala	Mbala Central	08.70408°	031.24962	MB04	Chizombwe
Northern	Mbala	Mbala Central	08.69394°	031.32022°	MB05	Mindolo
Northern	Mbala	Mbala Central	08.69280°	031.53506°	MB06	Mindolo
Northern	Mbala	Mbala Central	08.71437°	031.52115°	MB07	Kakondo
Northern	Mbala	Mbala Central	08.71383°	031.52251°	MB08	Kakondo
Northern	Mbala	Mbala Central	08.68584°	031.48838°	MB09	Kaluluzi/Kasesha
Northern	Mpulungu	Kaizya	08.77002°	031.11371°	MP01	Samaras
Northern	Mpulungu	Kaizya	08.78934°	031.11479°	MP02	Musende
Northern	Mpulungu	Kasimango	08.83453°	031.12314°	MP03	Maisunge
Northern	Mpulungu	Kasimango	08.83190°	031.12142°	MP04	Maisunge
Northern	Mpulungu	Isoko	08.78493°	031.26133°	MP05	Muswilo
Northern	Mpulungu	Isoko	08.78362°	031.26195°	MP06	Muswilo
Northern	Mpulungu	Isoko	08.78313°	031.26263°	MP07	Muswilo
Muchinga	Nakonde	Ilola	09.32963°	032.50304°	NK01	Katete
Muchinga	Nakonde	Ilola	09.34645°	032.52042°	NK02	Matuwi
Muchinga	Nakonde	Nteko	09.28826°	032.44727°	NK03	Nteko
Muchinga	Nakonde	Nteko	09.26414°	032.40359°	NK04	Musiani
Muchinga	Nakonde	Nteko	09.26270°	032.39831°	NK05	Musiani
Muchinga	Nakonde	Ntantumbila	09.17765°	032.36871°	NK06	Isansa
Muchinga	Nakonde	Ntantumbila	09.17963°	032.37656°	NK07	Londaila
Muchinga	Nakonde	Ntantumbila	09.14030°	032.34621°	NK08	Michembo

Province	District	Veterinary Camp	Latitude	Longitude	Respondent Id	Village
Muchinga	Nakonde	Ntantumbila	09.15108°	032.36740°	NK09	Uzinji
Muchinga	Nakonde	Ntantumbila	09.15228°	032.36826°	NK10	Uzinji
Muchinga	Nakonde	Nteko	09.26370°	032.40413°	NK11	Kangwa
Muchinga	Nakonde	Ilola	09.28304°	032.55467°	NK12	Movu
Muchinga	Nakonde	Ilola	09.28247°	032.55527°	NK13	Movu
Muchinga	Nakonde	Ilola	09.28444°	032.55872°	NK14	Movu
North-western	Solwezi		12°23.411	026°08.616	HH	Kaumba
North-western	Solwezi		12°29.613	026°11.458	CM	Chief Mumena area
North-western	Solwezi		12°23.537	026°14.345	JN	Chief Mumena area
North-western	Solwezi		12°24.944	026°13.653	PF	Chief Mumena area