ASSESSMENT OF THE EPIDEMIOLOGICAL STATUS,SEROPREVALENCE AND MOLECULAR DETECTION OF *PESTE DES PETITS RUMINANTS* IN GOATS AND SHEEP ALONG TANZANIA-MALAWI BORDER

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ABSTRACT

Peste des petits ruminants (PPR) is a highly contagious viral animal disease that impact negatively on food security of livestock keepers in Africa due to its fast spread and death of small ruminants. The disease was first reported in West Africa and afterwards Asia, rest of Africa including East Africa and Southern African Development Community (SADC) region. Lack of vaccination and effective application of diagnostic technologies to identify carriers has led to fast spread of the disease. Malawi, Zambia and Mozambique have been warned to be at high risk of infection of PPR due to their proximity to Tanzania and Democratic Republic of Congo (DRC), where the disease has been confirmed (FAO/OIE, 2015). Quantification of the disease status in these high risk countries will ensure effective surveillance and control to keep the disease from spreading. The aim of the present study was to provide epidemiological and molecular status of Peste des PetitsRuminants Virus (PPRV) in small ruminants along Tanzania-Malawi border. Whole blood (n=350), serum (n=350) and nasal swabs (n=100)were collected from goats and sheep in Kyela and Ileje (Tanzania) and Karonga and Chitipa(Malawi). A questionnaire was administered to 113 household heads to depict factors leading to spread of the disease. Molecular diagnosis was done by partial amplification of PPRVgenome using reverse transcription polymerase chain reaction (RT-PCR). Data analysis using chi square test for seroprevalenceand partial logistic regression to check for factors associated with seropositivity were carried out using Epi info statistical package. Based on the results, overall seroprevalencewas 11.1% and analysis showed that 31 out of 83small ruminants (37.3%) in Kyelawas significantly higher (p=0.000) than 8 out of 84 (9.5%) in Ileje. A total of 2 out 95 (2.1%) animals inChitipa had PPRV detected by RT-PCR. However, the PCR products did not yield any nucleotide sequence because the viral load was low. Communal grazing and free roaming husbandry practices were shown to be risk factors for the spread of PPR in these border

districts. The interviewed farmers had limited knowledge of the disease although they are helped by Veterinary and field officers for other diseases. It is concluded that there is no active infection of PPR along the border districts of Tanzania and Malawi, however low virus load may be present in Chitipa. It is recommendedthatappropriate strategies should be applied to prevent contact with infected animals through vaccination against PPRVon both sides of border districts.

DECLARATION

I, GladsonChikaikoKamwendo, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of my registration and that it has neither been submitted nor being concurrently submitted for a degree award in any other institution.

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thank the Almighty God for all the favors I have seen throughout the course of this study

and many more to come.

DEDICATION

I dedicate this work to my mum and dad for the encouragement they have shown to me. Also to my sisters and brothers: Naomi, Effie, Thokozani, Margaret, Precious and Stuart for the love and endurance during my absence throughout the 2 years of my studies. To my wife, Sungani for being there for me always.I love you all.

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LIST OF ABBREVIATIONS AND ACRONYMS

BD   Bacterial diseases

CCPP   Contagious caprinepleuropnemonia

C-ELISA Competitive enzyme-linked immunosorbentassay

CG   Communal grazing

DNA   Deoxyribonucleic acid

EDTA   Ethylene-di-amine-tetra-acetic acid

ELISA   Enzyme-linked immunosorbentassay

EMPRESS Emergency Systems for TransboundaryAnimal and Plant Pests and Diseases

EPA   Extension planning area

FA   Farmers with animals

FAO   Food and Agriculture Organization

FO   Field officer

HI   Helminthes infestation

MLFD   Ministry of Livestock and Fisheries Development

ND   No diseases

NPK   No peste des petits ruminants knowledge

OD   Optical density

OIE   World Organisation for Animal Health

OR   Odds Ratio

PK   *Peste des petits ruminants* knowledge

PLS   *Peste des petits ruminants* like signs

PPR   *Peste des petitsruminants*

PPRV   *Peste des petitsruminants*virus

RNA	Ribonucleic acid
RT-PCR	Reverse transcriptionpolymerase chain reaction
SADC	Southern African Development Community
ST	Self treatment
T	Tethering
TADs	Transboundary Animal Diseases
TAE	Tris-acetate ethylene-di-amine-tetra-acetic acid
UV	Unvaccinated
V	Vaccinated
VO	Veterinary officer

CHAPTER ONE

1.0   INTRODUCTION

1.1   Background Information

Small ruminants production contributes significantly to food security across African communities but frequent attack by fatal diseases has brought a big challenge. Over the years, new infectious diseases and more contagious diseases have emerged which pose a threat to livestock industry. These diseases are capable of fast spreading to other areas and are called transboundary animal diseases (TADs). The Southern Africa Development Community (SADC) region has not been spared of such diseases especially those attacking small ruminants like *peste des petits ruminants* (PPR).*Peste des petits ruminants* can cause direct economic losses to livestock keepers and affects the socio-economic status of African communities (Kihu*et al*., 2015). Small ruminants (sheep and goats) contribute about 22% to the national meat supplies of Tanzania. Sheep and goat meat production is being practiced by about 30% of the agricultural households in Tanzania (MLFD, 2011). The ability to multiply and grow faster than cattle, at relatively low cost makes shoats more attractive to small-scale farmers. Prevalence of diseases has been one of the major constraints for low productivity in these species.

*Peste des petits ruminants*is a highly contagious animal disease affecting domestic goats and sheep as well as some wild ruminants (Munir*et al*., 2012). The disease is caused by the *peste des petitsruminants*virus (PPRV), which belong to *morbillivirus* group of the *paramyxoviridae* family of viruses. The virus is closely related to rinderpest virus of cattle and buffaloes, measles virus of humans, distemper virus of dogs and some wild carnivores and morbilliviruses of aquatic mammals. Rinderpest is the first animal virus to be eradicated in the world. *Petse de petits ruminants* has affected most parts of Africa, with

East, Central and West Africa being the endemic foci and it's imposing a high risk of spread towards the south of Africa. The disease has been reported in north east Uganda (Mulindwa*et al*., 2011), Kenya(Luka *et al*., 2012), Democratic Republic of Congo (FAO, 2012), northern, eastern and southern Tanzania (Karimuribo*et al*.,2011;Muse *et al*., 2012;Kgotlele*et al*., 2014) and hence pose a threat of possible spread to Southern Africa including Malawi.Therefore, the study assessed the epidemiological status, seroprevalence and molecular detection ofPPR along the Tanzania-Malawi border districts.

1.2   Problem Statement

Transboundary animal diseases have devastating impact on the survival of small ruminants and also economic, food security and livelihoods of poor people across the globe (Banyard*et al*., 2010). A disease like PPR has a potential of spreading rapidly irrespective of continental, regional or national borders. PPR is considered one of the damaging animal diseases in Africa, the Middle East and Asia(FAO-OIE, 2015). The SADC region is under threat of the spread of PPR introduction with possibility of reaching other countries within the region.

Lack of interest paid to the disease since its discovery is largely responsible for its spread (Libeau*et al.*, 2011). The global distribution of the disease, although expanding relentlessly, is fairly known (FAO-EMPRESS, 2012). Quantification of disease status is one of ways to ensure effective surveillance and control to keep the disease from spreading. Application of diagnostic techniques such as serology for screening coupled with the knowledge of risk factors associated with the distribution of the PPR can be used to sensitize the public and other practitioners on possible ways to manage the disease.

1.3   Justification

Seroprevalence and molecular biology as well as epidemiological factors associated with possible spread of PPR in some parts of south west Tanzania have not been researched to provide evidence of the absence or present of PPR. Therefore, the present study will providethe information on the epidemiological, seroprevalenceand molecular status of PPRV in the border area of Tanzania and Malawi to be used for effective control measures for the disease.

1.4   Research Objectives

1.4.1   Main objective

To assess the epidemiological status, seroprevalence and molecular detection of PPR along the Tanzania-Malawi frontier.

1.4.2   Specific objectives

(i)   To determine the seroprevalence status of PPR in goats and sheep,

(ii)   To determine the epidemiological factors associated with PPR spread in goats and sheep

CHAPTER TWO

2.0   LITERATURE REVIEW

2.1   TransboundaryAnimal Diseases (TADs)

Transboundary animal diseases(TADs) are those diseases of significance economic, trade and food security importance for a considerable number of countries which can easily spread to other countries and reach epidemic proportions and where control or management of the disease requires the corporation of several countries (Otte*et al*., 2004). Transboundary animal diseases are epidemic diseases that are highly contagiousand can spread fast regardless of national borders. These diseases cause a high morbidity and mortality in susceptible animal populations that can lead to severe economic, animal and possibly public health consequences (FAO, 2012). Most developing countries are having difficulties coping with TADs due to lack of adequate resources. The resource constraint problem has resulted in disease endemicity posing a threat to food security as well as increased spread of disease via animal trade (Rossiter, 2009). It has been suggested that effective means by which transmission and outbreaks of these diseases can be prevented and controlled are by efficient control of import of animals and animal products, control of animal movement, surveillance for TADs, emergency plans, veterinary services, access to vaccines for use if required and adequate laboratory support (Rossiter, 2009). One of TADs that is spreading to the south of SADC region is PPR.

2.2   PesteDes Petits Ruminants Virus

2.2.1   Virusclassification

Peste des petitsruminants disease is caused by PPRV, avirus classified into the family *Paramyxoviridae*and the genus *Morbillivirus* (Gibbs *et al*., 1979). The other members of this group are measles, canine distemper andrinderpest which infect humans, dogs and

cattle, respectively.Rinderpest has already been eradicated (OIE, 2011). *Petse des petits ruminants* is a list A disease, a major notifiable disease of the World Organisation for Animal Health (OIE).

2.2.2   The virion

PPRvirion is a spherical enveloped particle having fusion proteins (F) and haemagglutinin (H) on the surface (Fig. 1). The matrix protein (M) inside the envelope stabilizes virus structure and surrounds the viral RNA which is composed of nucleoproteins (N). The N protein surrounds the genomic RNA along with other viral proteins, polymerase proteins (L) and phosphoproteins (P) (Barrett *et al*., 2006; Munir*et al*., 2013).

2.2.3   Genome structure of PPRV

PPR viral genome is a linear, single stranded, non-segmented, negative sense RNA with 15948 nucleotides. PPRV genome carries six transcriptional genes which encode six polypeptides namely nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), haemagglutinin protein (H) and polymerase protein (L) (Luka *et al*., 2011; Munir*et al*., 2013) (Fig. 2). All these genes are arranged in an order of 3'-N-P/C/V-M-FHN-L-5' (Bailey *et al*., 2005).
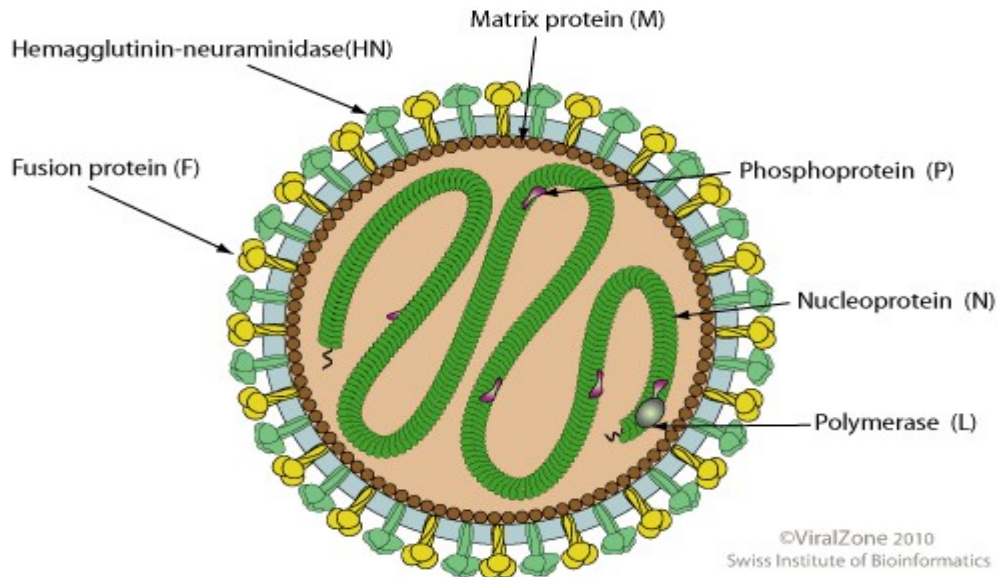
Figure 1:   *Peste des petitsruminants*virion structure. The virion is composed of fusion protein (F), haemagglutinin-neuraminidase (HN), viral RNA (nucleoprotein) surrounded by matrix protein (M) and its associated phosphoprotein (P) and polymerase (L). Source: Viral zone (2010).
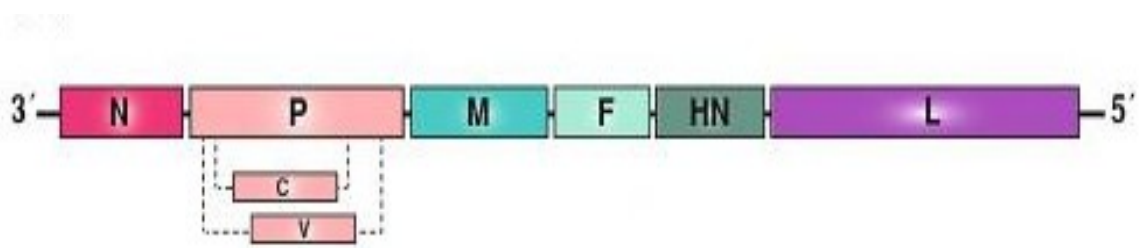
Figure 2:   PPRV genome containing eight open reading frames.The genome containsnucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), haemagglutinin protein (H) and polymerase protein and non-essential genes C and V. The genome is negative sense and is converted into mRNA by a viral RNA-dependent RNA polymerase before translation of viral protein can occur. Source: Munir*et al*. (2012).

2.2.4   Molecular characterization of *Peste des petits ruminants*

Phylogenetic characterization of PPRV shows four distinct lineages namely; I, II, III and IV that are distributed based on geographical locations (FAO, 1999).   Since PPR discovery in 1942 in Ivory Coast, Lineage I and II have been reported in West Africa.Central Africa (Gabon and Cameroon) and North Africa (Morocco) have been reported to have lineage IV (Kwaitek*et al*., 2011; Sanz-Alvarez *et al*., 2008). Recently in east African countries, Uganda, Kenya and Tanzania lineages II, III and IV have reported (Kgotlele*et al*., 2014; Dundon*et al.,* 2015; Banyard*et al*.,2015). In addition to lineage III circulating in east Africa, lineages II and VI have been reported in Uganda and Tanzania (Luka *et al*., 2012; Misinzo*et al.,* 2015).

2.3   Epidemiology of PPR

Peste des petits ruminant is primarily a disease of goats and sheep. However, the virus has been isolated in other animals like camels (Khalafalla*et al*., 2010), cattle (Lembo*et al*., 2013 and Özkul*et al*., 2002) and gazelle (Sharawi*et al*., 2010). These species can be of value for PPR sero-surveillance. The disease has been shown to have high morbidity and mortality in naive populations of goats and sheep. Mortality can reach up to 90% (Zahur*et al.,* 2009) in lambs and kids with goats being more affected than sheep (Chauhan*et al.*, 2009). Periodic outbreaks may be experienced when animals are mixed or new animals are introduced into a herd (Muse *et al*., 2012) as well as purchase animals from live animal market. Recent spread of PPR in Africa has been correlated with increased animal movement for commercial and trade purposes, nomadic customs and extensive farming practices in the Saharan regions (FAO, 2013). Climatic factors have been shown to be favoring the survival and spread of the virus (Mahajan*et al*., 2013). In endemic situation it is expected that very young kids would be protected by maternal antibodies, however, goats under one year of age can show highest morbidity and mortality (Rahman*et*

*al.,*2011) probably due to reduced immunity from the dam. The risk of PPRV introduction in an area can be a function of the probability of hazard (PPRV) release, exposure of susceptible hosts and the consequences of spread using prevalence of infection, volume of trade, competitive enzyme linked immunosorbent assay(c-ELISA) missing an infected animal (Chazya*et al.,* 2014). Several risk factors can be associated with PPR spread from one area to the other (Kihu*et al*., 2012). Knowledge of the epidemiology of PPR is essential to effectively develop control programmes for the prevention of the disease.

2.4 PPR Transmission and Clinical Diagnosis

2.4.1 Transmission

Peste des petits ruminants is a fragile virus that requires close contact between animals for its transmission (Sigh, 2011). Discharges from eyes, nose and mouth contain large amounts of the virus. Most infections occur through inhalation of the aerosols from sneezing and coughing (Roeder *et al*., 1999; FAO, 1999). This can be possible in circumstance where animals are brought to central local markets, migratory flocks and common grazing lands.

2.4.2 Clinical signs and post-mortem findings

Peste des petits ruminants is diagnosed preliminary by common clinical signs of high fever (41$^{\mathrm{o}}$C), depression, dehydration, anorexia, severe purulent lacrimation resulting into reddening of conjunctiva and matting of the eyelids, severe purulent nasal discharges, respiratory distress, coughing and oral lesions (Gitao*et al*., 2012). Sharawi*et al.* (2010), reported to have seen catarrhal nasal discharges and crusts around the nostril, localized lesions on the lips, soft palates consisting of gray to yellow pseudo membranous foci in clinically ill goats and sheep. Post-mortem lesions similar to rinderpest are usually observed. Bronchopneumonia with consolidation of the liver occurs frequently.

Congestion and enlargement of the spleen is often seen and also the lymph nodes are generally congested, enlarged and edematous.

2.4.3   Differential diagnosis

Peste des petits ruminants are frequently confused with diseases that present fever and grossly similar clinical signs, especially when it is newly introduced. *Peste des petits ruminants*should be differentiated fromrinderpest, foot-and-mouth disease, bluetongue or contagious ecthyma (orf), pneumonic pasteurellosis, contagious caprinepleuropneumonia (CCPP) and coccidiosis or gastro-intestinal helminth infestations. However, pneumonia is usually a very obvious presenting sign inPPR so, without doubt, pneumonic pasteurellosis andCCPP have caused the most difficulty in differentialdiagnosis (FAO, 1999).

2.5   Laboratory Confirmation

2.5.1   Enzyme-linked immunosorbent assay

Conventional serological tests such as agar gel immunodiffusion (AGID), haemaglutination(HA) and enzyme-linked immunosorbentassay (ELISA) have been used but lack sensitivity compared to other tests (Abubakar*et al*., 2012; Ularamu*et al*., 2012). However, a monoclonal antibody based c-ELISA has been recommended for detection of the prevalence of infection and disease surveillance in both individual and population due to its sensitivity, specificity and reliability (OIE, 2013). It has also been demonstrated to be viable screening test compared to virus neutralizing test (VNT) (Singh *et al.,* 2004; Rahman*et al*., 2011) and more sensitive for PPR sero-surveillance if the target population is non-vaccinated.

2.5.2 Reverse transcription polymerase chain reaction

Detection of PPRV can confirm the PPR diagnosis in the event of an outbreak with well-presented clinical signs. In areas where mass vaccination of sheep and goats against PPR

is being done accordingly, detection of the antibodies alone can yield false prevalence (Mehmood*et al*., 2009). Thus in such circumstances, detection of the virus in clinical samples becomes vital to ascertain the presence of the PPRV. Diagnostic tools based on viral genome detection such as reverse transcription polymerase chain reaction (RT-PCR) have been used to detect the virus. The tool works by first copying the RNA into DNA using reverse transcriptase followed by amplification of DNA in a two-step reaction known as RT-PCR. These genome based techniques are used because of their high sensitivity and specificity, however, for PPRV a one-step real time RT-PCR is more sensitive (Abubakar*et al*., 2012). Some studies have combined RT-PCR with nucleotide sequencing to determine the interrelationship of different lineages of PPRV to member of same genus and family (Chauhan*et al.,* 2009). Knowledge of these relationships among different lineages can be used for control, prevention and in long run for eradication of the diseases in different parts of the world.

2.6   PPR Disease Control

Currently there is no treatment for PPR in small ruminants. The control of PPR in endemic areas can be achieved by vaccination. Animals that recover develop good immunity, which persists for at least four years and possibly lifelong. Control of PPR outbreaks mostly relies on movement control (quarantine) combined with ring vaccination and prophylaxis immunization in high risk populations (OIE, 2008).

2.7   Geographical Distribution of PPR

2.7.1   World distribution

Petse des petits ruminants was first discovered in 1942 in Ivory Coast. However, since 2008, most parts Africa have been infected with the disease. *Peste des petits ruminants* endemic zones include the countries located between the Sahara and Equator, from the

Atlantic Ocean to the Red Sea. The disease has also been reported in Middle and Far East countries and in the Indian (Sanz-Alvarez, 2008) subcontinent (Pakistan, India, Bangladesh, and Nepal). From West Africa, PPR quickly spread to Central African, East Africa, the Middle and Far East as well as Asian countries like China (Geerts, 2009).

2.7.2 PPR in Tanzania

Tanzania was officially declared to be infected with PPR in 2008 and it was believed to have come from Kenya due to the pastoralism, practiced by communities along these borders (Karimuribo*et al.,* 2009). A separate study done in 2010 by Kivaria*et al*(2013) indicated that one of the sources of infection to be from northern neighboring countries. Confirmed presence of PPR in southern Tanzania (Muse *et al*., 2012) and preliminary investigation suggesting the presence of the PPR in Morogoro (Kgotlele*et al.,* 2014) suggests a fast spread of the disease to some parts of Tanzania. A high risk of introduction of the disease into Zambia was observed (Chazya*et al*., 2014) due to cross border trade of small ruminants on the western side of Tanzania-Zambia borders.

2.7.3 PPR in Malawi

Malawi is considered free from PPR and at high risk of infection from the neighbors. *Peste des petits ruminants* being a transboundary animal disease, there is need for neighboring countries to be alerted from those affect and international community to be informed of its presence or absence (Wambura, 2000) in order to achieve effective diagnosis and surveillance for timely control.

2.8 Goats and Sheep Population in Tanzania and Malawi

2.8.1 Tanzania

Livestock sector contributes significantly to the livelihoods of the agricultural community in Tanzania. The main types of livestock raised by farmers in Tanzania are cattle, goats,

sheep, pigs, chickens, ducks, turkey and rabbits. The total population for goats and sheep is 15154121 and 5715549 with 15085150 and 574 found in mainland Tanzania and Zanzibar, respectively. In Mbeyawhere the study was conducted has a total of 98222 sheep and 544469 goats (Livestock Sector- National Report, 2012). Kyeladistricthas 4869 goats and 1369 sheep and Ileje district has 32 210 goats and 5942 sheep (MbeyaRegional Secretariat, 2013).

2.8.2   Malawi

Livestock at household level is important for food security as a source of food and asset. The main types of livestock are cattle, goats, sheep, pigs and chickens. The total number of goats and sheep is 2623017and 76713, with 420964 goats and 2017 sheep in the northern region, respectively. Chitipa district has 28736 goats and 1686 sheep while Karonga has 16537 goats and 331sheep (NSO, 2010).

CHAPTER THREE

3.0   MATERIALS AND METHODS

3.1   Study area

The study was carried out in four districts along the border of Tanzania and Malawi namely Kyela and Ileje, and Karonga and Chitipa which are located in the South Western side of Tanzania and Northern side of Malawi, respectively (Fig. 3). Tanzania is located in the Eastern side of Africa which lies between latitudes 1^0 and 12^0 south of the equator and 29^0 and 41^0 east of the Greenwich meridian. The bordering countries are Kenya, Uganda to the north, Burundi, Rwanda and Democratic Republic of Congo (DRC) to the West, Zambia and Malawi in the South West and Mozambique to the south. Indian Ocean is in the East side of Tanzania. The mainland of Tanzania is $942784km^2$ and 39% of the total land is used for grazing. Malawi lies between latitudes 9^0 and 18^0 south of the equator and longitudes 32^0 and 36^0 east of Greenwich meridian. It occupies the strip of land between Zambia to the North West, Mozambique in the South and Tanzania in North East. It has total land of $94080km^2$.
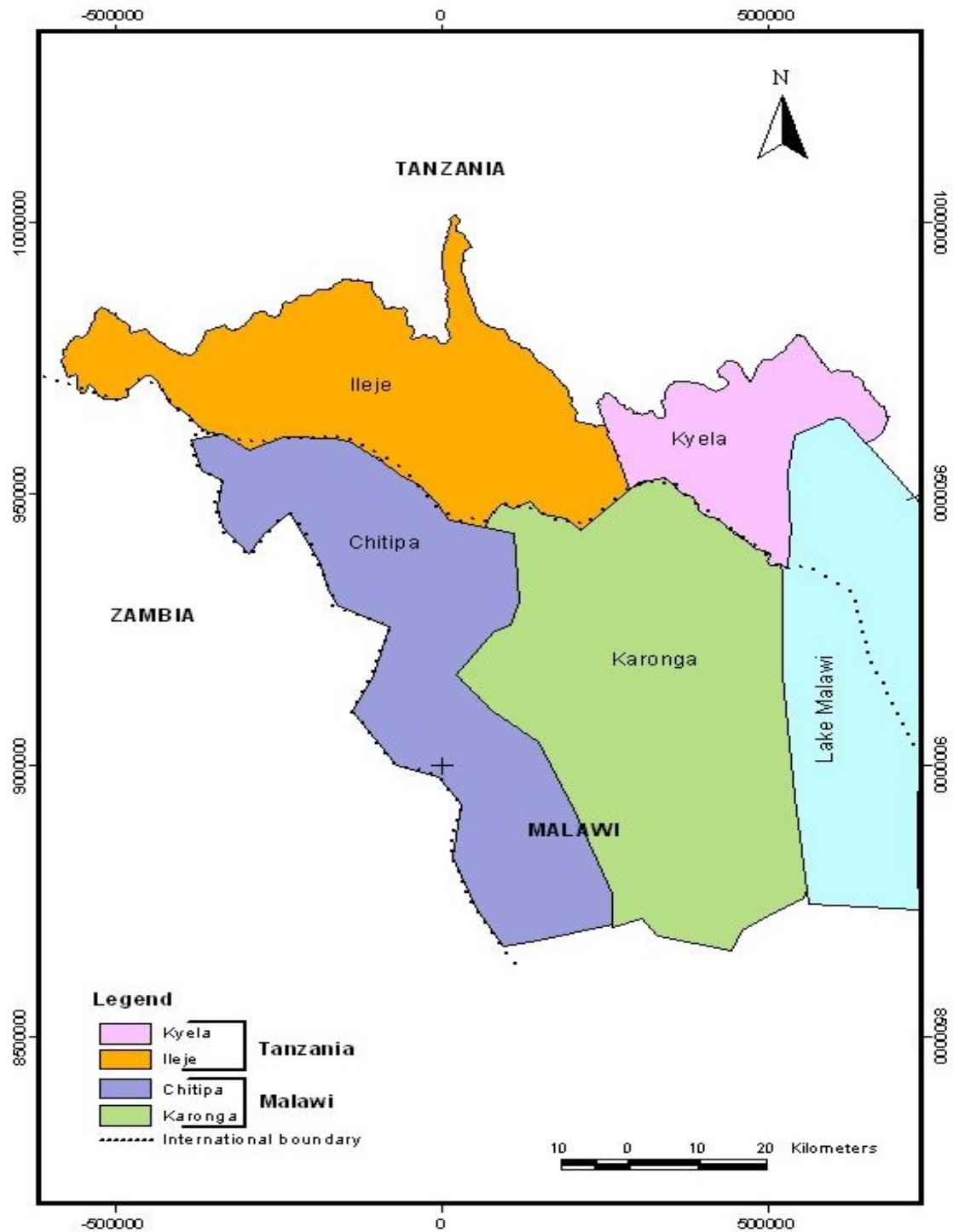
Figure 3: Map of the study area along the Tanzania and Malawi border.Samples from goats and sheep were obtained from Kyela and Ileje districts ofTanzania and Karonga and Chitipa districts of Malawi.

3.2   Determination of Sample Size

The sample size was calculated using the formula below, previously described byDohoo*et al*. (2009).

$$n = Z^2_{\alpha(0.05)} \times \left[\frac{p(1-p)}{L^2}\right] \qquad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(1)$$

Where z =1.96, p = 0.32 (prior prevalence from earlier studies) and L = 0.1 (the desired level of precision or accuracy). Therefore, the sample size was comprised of 84 households. A mark-up value of 5% was included to make the sample to be 88 households.

3.3   Research design and Data Collection

A cross-sectional study design was deployed in order to obtain data from the villages along the border of Tanzania and Malawi.Wards and extension planning areas (EPAs) along the border from the Tanzanian and Malawi districts were purposively selected respectively. Villages along the border were selected and total of 113 households from the four districts participated in the study. At least 2 to 3 animals (goats or sheep) per household were sampled and these were in the age range of less than two year and more than six months. A well-structured questionnaire (Appendix 1) was administered to each livestock keeping household head selected, from whose animal samples were taken. The questionnaire focused on information about the flock size, location, species, sex, health, vaccination status and management practices, knowledge about the disease, veterinary services and diseases being experienced apart from PPR. Knowledge about PPR was indicated that the household head can clearly distinguish the signs from other diseases occurring in the area. Health status data was documented by recording history any of disease outbreak or occurrence.

3.4   Sample Collection and Preparation

3.4.1   Collection of sera for serological analysis

Blood samples from goats and sheep were collected from the jugular vein using four ml plain vacutainer tubes (Shanghai International Holdings Corp. GmbH, Hamburg, Germany) and needle. The blood was left to clot overnight at room temperature. Serum was decanted into 1.5 mlsterile cryovials and transported on ice to the laboratory. The tubes were labeled using codes describing the villages from which the samples were taken. The sera were stored at -20^{0}C until analysis.

3.4.2   Collection of samples for molecular analysis

3.4.2.1 Whole blood

Blood samples from goats and sheep were collected from the jugular vein using four mlvacutainer tubes (Shanghai International Holdings Corp. GmbH, Hamburg, Germany) containing anticoagulant, ethylene-di-amine-tetra-acetic acid (EDTA) and needle. The tubes were labeled using codes describing the village from which the samples was taken. The samples were transported on ice to the laboratory. The buffy coat was obtained by centrifuging the blood in EDTA tubes at 6000gfor five minutes at room temperature. The whitish middle layer of the centrifuged whole blood between plasma and erythrocytes (buffy coat) was harvested and transferred into a sterile cryovials and stored at -20^{0}C until RNA extraction.

3.4.2.2 Nasal swabs

Nasal swabs were collected from sheep and goats and placed in a three ml Universal viral transport medium (Shanghai International Holdings Corp. GmbH, Hamburg, Germany). These were mixed thoroughly by shaking vigorously to dislodge the cells from the swab

into the media. The samples were transported on ice to the laboratory. The mixture was transferred into a sterile cryovials and stored at -20^{0}C until RNA extraction.

3.5   Sample Analysis

3.5.1   Serological detection of antibodies against PPRV

The serum samples were tested using a c-ELISA kit developed by IDvet innovative diagnostics (CIRAD-EMVT, Montpellier, France) according to Libeau*et al.* (1995)(Appendix 2). Briefly the 96-well micro plateswere already coated with PPR recombinant nucleoprotein antigen to which the test sera, controls (positive and negative) and diluent buffer were added and incubated at 37^{0}C for 45 minutes. Afterwards, an anti-nucleoprotein horseradish peroxidase (Anti-NP-HRP) conjugate was added followed by substrate. Color was allowed to develop for 15 minutes followed by reading of plates at 450 nm in a spectrophotometer (MTX Lab systems, Vienna, USA).

3.5.2   Detection of PPRV genome by RT-PCR

3.5.2.1 RNA extraction

Viral RNA was recovered from the buffy coat and nasal swabs according to manufacturer's instructions using the Pure linkviral RNA/DNA mini extraction kit (Invitrogen, Carlsbad, CA) (Appendix 3). Briefly, the samples were lysed using lysis buffer with 2-mercaptoethanol and the precipitate were dissolved using ethanol. Proteins were pelleted by centrifugation and the lysate was then passed through a column followed by washing and cleansing of bound RNA. The RNA was then eluted with RNase free water. The extracted RNA was stored at -20^{0}C until purification.

3.5.2.2 Detection of PPRV by RT-PCR

Reverse transcription polymerase chain reaction was carried out in GeneAmp PCR System 9700 (Applied Biosystems, Carlsbad, USA). The master mix was prepared (Table 1) using AgPath-ID one step RT-PCR kit (Applied Biosystems,Carlbad, USA)with PPRV specific primers (NP3 and NP4).

Table 1: Reverse transcription polymerase chain reaction master mix components

Component	Volume (μ)
2X RT-PCR Buffer	12.5
Nuclease-free water	8.5
Forward primer (10μM)	1.0
Reverse primer(10μM)	1.0
RT-PCR Enzyme (Reverse transcriptase)	1.0
Extracted RNA template	1.0
Total volume per reaction	**25.0**

3.5.2.3 Gel electrophoresis and visualization

Reverse transcription polymerase chain reaction products were separated by electrophoresis on a 1.5% agarose gel in 0.5 %Tris-acetate Ethylene-di-amine-tetra acetic acid (TAE) buffer (SERVA Elactrophoresis, Heidelber, Germany) stained with GelRed nucleic acid stain (Phenix Research Products, Candler, USA). Each well was loaded with 5μl of the PCR product and 1μl of blue/orange 6X DNA loading dye (Promega, Madison, USA). The samples were separated along with a 1000bp DNA ladder at 100 Volts for 30 minutes. The agarose gel was visualized by ultraviolet fluorescence light using a UV trans-illuminator(BIO RAD, Gel doc EZ imager)which was connected to a computer to document the agarose gel pictures.

3.5.3 N-gene sequencing and phylogenetic analysis

The PCR products obtained after an RT-PCR targeting the N-gene were sequenced using NP3 and NP4 primers using automated di-deoxynucleotide sequencing. The sequences were manually assembled and aligned using cluster W along with other sequences representing the four lineages of PPR. Afterwards, phylogenetic analysis was performed using the minimum evolution method with 1000 bootstrap replications as implemented in MEGA 6.0.

3.6   Data Analysis

Data obtain from the villages, livestock keepers and individual animal was stored in Microsoft Excel 2010. Descriptive statistics for the animal and flock level explanatory variables examined was used to calculate proportions for seroprevalence and factors that include animal species, sex, disease knowledge and feeding systems. Regression analysis was conducted to compute the correlation been risk factor and presence of the disease. Statistical significance for the proportions was compared using Chi-square test in Epi Info software version 7 (Centre for Disease Control and Prevention, Atlanta, Georgia). A confidence limit of less than 5% was used to indicate a significant level.

CHAPTER FOUR

4.0   RESULTS

4.1   Small Ruminants Herds

Interviews were performed on 113 household heads from four districts including Chitipa and Karonga in Malawi and Ileje and Kyela in Tanzania. A total of 350 small ruminants (four sheep and 346 goats) out of 625small ruminants in the study area(31 sheep and 594 goats) were sampled for whole blood and sera (Table 2). The average herd size in the study area was found to be 5.5 animals per households (range 1 to 26 goats).

Table 2:   Distribution of small ruminants among the households visited in Chitipa, Karonga, Ileje and Kyela along the Tanzania-Malawi border

District	Interviewed Households	Number of Animals			Sampled Animals			Average herd size
		Goats	Sheep	Total	Goats	Sheep	Total	
Chitipa	26	180	28	208	93	2	95	8.0
Karonga	31	155	0	155	88	0	88	5.0
Ileje	23	157	1	158	84	0	84	6.9
Kyela	33	102	2	104	81	2	83	3.2
Total	**113**	**594**	**31**	**625**	**346**	**4**	**350**	**5.5**

4.2   Seroprevalence of PPR in Small Ruminants

A total of 350 sera were analyzed from the four districts using a cELISA to detect antibodies against PPRV (Table 3). The overall seroprevalence was 11.1% in small ruminants of Ileje (9.5%) and Kyela (37.3%) districts. Doubtful seropositivitywas found in Karonga, Ileje and Kyela. ThePPRantibodies were not found in sera collected from small ruminants in Chitipa.

Table 3:   Seroprevalence of PPR in small ruminants at border districts of Tanzania and Malawi

District	Samples tested			Samples positive	Doubtful	Seropositivity (%)	X^2(p-value
	M	F	Total				
Chitipa	4	91	95	0	0	0	
Karonga	6	82	88	0	3	0	
Ileje	6	78	84	8	7	9.5	
Kyela	3	80	83	31	1	37.3	16.5 (0.00)
Total	**19**	**331**	**350**	**39**	**11**	**11.1**	

4.3   Molecular Diagnosis of PPRV Using RT-PCR

Reverse transcription polymerase chain reaction was run on 350 blood samples and 100 nasal swabs from a total of 350 of small ruminants.*Petse des petits ruminants* virus was detected in two out of 95 (2.1%) blood samples collected in Chitipa and tested using the PPRV primers NP3 and NP4 (Fig. 5). These two positive samples were from sheep and the other from goat. The samples from Karonga, Ileje and Kyela did not show any presence of the PPRV from both blood and nasal swabs. The two positive samples for PPRV using the RT-PCR had low quantities of PCR products and could not be sequenced.
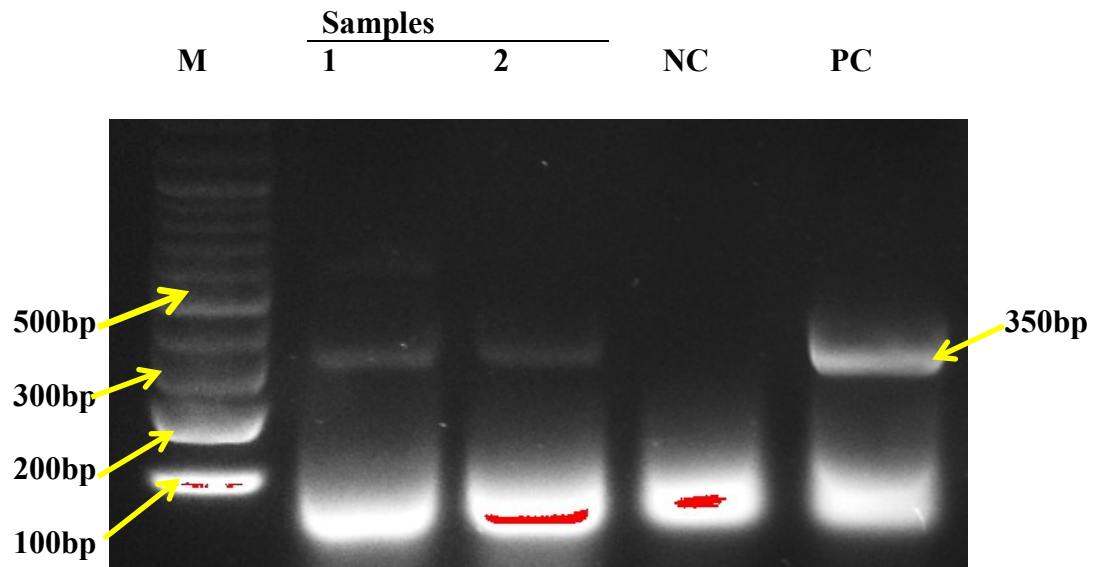
Figure 4: Detection of PPRV using RT-PCR as visualized under UV transilliminator.Bloodand swabs from goats and sheep collected in Chitipa were tested using RT-PCR. M is the DNA marker and the RT-PCR was performed using primers NP3 and NP4 producing an expected band size of 350bp. The 350bp band is also seen in positive control (PC) and in positive samples (1 and 2) but not in negative control (NC).

Table 4: Distribution of PPRV as detected by the RT-PCR

District	Sample type	Sample tested	Sample positive	%positive
Chitipa	Whole blood	95	2	2.1
	Swabs	29	0	0.0
Karonga	Whole blood	88	0	0.0
	Swabs	21	0	0.0
Ileje	Whole blood	84	0	0.0
	Swabs	25	0	0.0
Kyela	Whole blood	83	0	0.0
	Swabs	25	0	0.0
Total	**Whole blood**	**350**	**2**	**0.6**
	Swabs	**100**	**0**	**0.0**

4.4 Risk Factors for PPR in Small Ruminants

The logistic regression was done to determine the risk factors that are associated with the PPR seroprevalence on small ruminants. Five factors were isolated,communal grazing feeding system, animal sex and PPR Knowledge showed an association with PPR seropositivity(Table 5). On awareness and practices of farmers in respect to PPR (Table 6) a detailed summary is indicated in Appendix 4.Most farmers in these four districts practice tethering systems (49%) of feeding their small ruminant than communal grazing (35%) and leaving the animal to roam (17%). Most farmers had not experienced any disease (55%) for the past three year with very few had an experience of helminths (40%), bacterial diseases (2%) and PPR like signs (4%).Eighty nine percent of farmers in these four districts indicated to have no knowledge on PPR while 11% know or heard the disease. Twenty three percent of the livestock farmers interviewed indicated to have vaccinatedtheirsmall ruminants against PPR in 2012 whilst 77% did not. These farmers indicated consulting veterinary officers (45%), field officers (50%), for any veterinary services. Other farmers do treat animals themselves (2%) while 3% consult fellow livestock keepers.

Table 5: Risk factor analysis associated with PPR seropositivity in border districts of Malawi and Tanzania.

Variable	Odds ratio (95% CI)	p-value
feeding systems		
communal grazing	10.35	0.000
roaming	0.18	0.000
tethering	0.91	0.760
animal sex (male and female)	1.06	0.925
animal species (goat and sheep)	0.49	0.546
PPR Knowledge (yes or no)	1.19	0.690
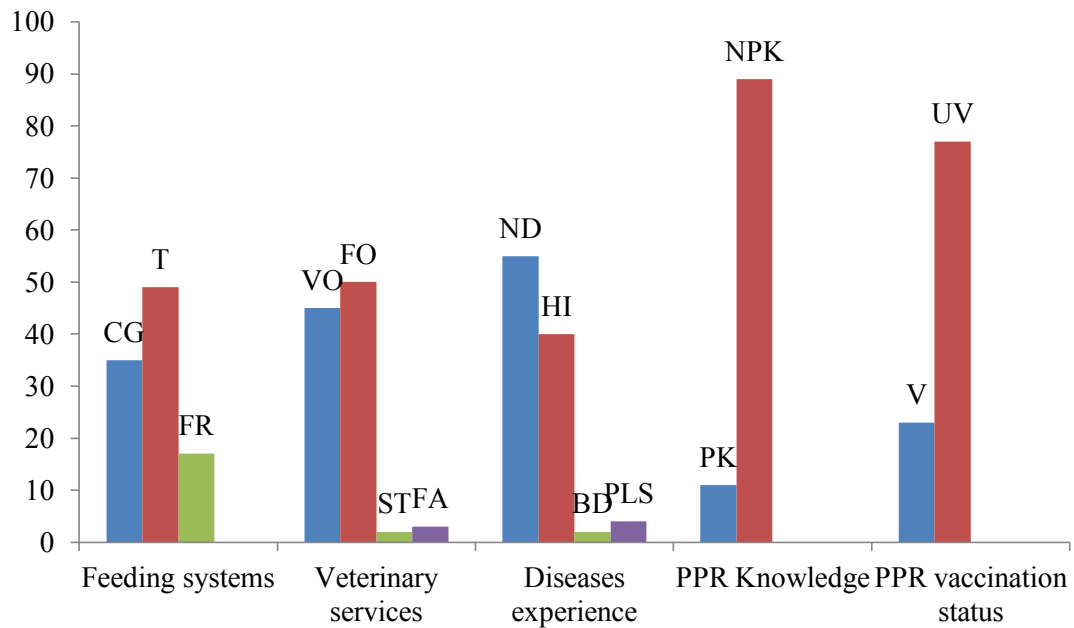
Figure 5: Practices and awareness by farmers with respect to PPR in border districts of Malawi and Tanzania.Percentages were computed in different categories of feeding system (CG = communal grazing, T = tethering and FR = free roaming), veterinary services (VO = veterinary officer, FO = field officer, ST = self-treating and FA = farmers with animals), disease experience in past three years (ND = no diseases, HI = helminthes infestation, BD = bacterial disease and PLS = PPR like signs), PPR knowledge (PK = PPR knowledge, NPK = no knowledge of PPR) and PPR vaccination conducted on small ruminants (V = vaccinated, UV = unvaccinated).

CHAPTER FIVE

5.0 DISCUSSION

Peste des petits ruminants is an emerging small ruminants disease and geographically spreading to new region across African continents. This study assessed the status of PPR along the Tanzania and Malawi border. *Peste des petits ruminants*being a transboundary disease, the presence or absence of the disease along this border districts is of essential importance to ensure proper control measures are put in place. At the time of the study, no outbreak was reported and no clinical signs were observed in the small ruminant population. Households keeping small ruminants were contacted and interviewed and samples collected for laboratory analysis.

Goats are one of the most kept small ruminants in the border districts of Tanzania and Malawi as shown in this study. Most of the goats and sheep are kept on a small scale with an average herd size of 5.5 in these communities with the highest average size of 8.0 being in Chitipa district followed by Ileje at 6.9%. The small ruminants herd size has been found to be 8 in Malawi (Chikagwa-Malungaand Banda, 2006) and 9 in Tanzania (MLFD, 2010) per goat keeping households. Theseanimals are threatened by increased immergence of infectious diseases like PPR and if not checked in these border areas may affect the farmers economically.In addition, there is a risk of spread of PPR further south of SADC region. This can be a result of the presence of the disease in Tanzania (Muse *et al*., 2012 and Misinzo*et al.*, 2015), Uganda (Lernfelt, 2013; Luka *et al*., 2012 and Mulindwa*et al.*, 2011) and Kenya (Kihu*et al.,* 2015). Malawi and Zambia have been reported not having the disease (FAO/OIE, 2015).

Surveillance and confirmation of PPR is mostly done in laboratories using serological tests like cELISA (Libeau*et al*, 1995). This study used cELISA to detect the antibodies against PPR virus in sera to estimate the presence of the virus in the area. However, seropositivityvaried in the different districts includingKyela (37.3%) and Ileje (9.5%) districts of Tanzania which differed significantly (p=0.000). The overall prevalence was 11.1%, which lower than in some studies conducted in Tanzania where seroprevalence of 31% (Muse *et al*., 2012) and 24.3% (Kgotele*et al*., 2014) were estimated.No small ruminants were found to be seropositive in Chitipa and Karonga. However, the presence of doubtful results even in Karonga where vaccination was not done can suggest movement of these animals across the border.

Detection of the antibodies alone can be misleading or some new infection can be missed, thus the use of molecular techniques like RT-PCR was used to see if there is an acute infection in the area. The test only detected two samples which were belonging to Chitipa district and these failed forcELISA antibody detection. It can be suggested that the animals have the virus which is just a new infection.However, the sequencing of these RT-PCR products did not yield any sequence because of a very low viral load. The communities in these border districts have some commonalities in terms of beliefs and relations as such movement of livestock is common. Some farmers have their relatives in either side of the boundary as such the two PPRV detected samples from Chitipa might have been brought in from Tanzania. It is important that the measures are taken to avoid further spread of the disease to Malawi from Tanzania.

Epidemiology of a disease can best be understood by looking at factors that can lead to introduction as well as spread of the disease. In this study, fivevariables were identified and only communal grazing system (OR =10.35) and free roaming animals (OR =0.18)

was associated with the seropositivity and significant (p=0.000) and is a possible way of PPR spread. Animals are more likely to be at risk of infection with PPR in communal grazing than free roaming. Salih*et al.* (2014) reported that open grazing system can be a risk factor for transmission of PPR in unvaccinated small ruminant's populations.

Out of the 113 farmers interviewed, communal grazing (96.8%) and tethering feeding system (91.3%) of small ruminants are mostly practiced in Karonga and Ileje respectively. In Kyela(48.5%) and Chitipa(65.4%) tethering was preferred than communal grazing. Baramurugan*et al.* (2011) reported that increases and widespread of virus infections like PPR can be attributed to goat and sheep husbandry practices as well as migration of livestock. The fact that these livestock keepers have small stock herd size per house, it's easier to tether the animals than graze them in communal areas which are also few. The tethering can ensure minimal contact between the flock hence enabling minimal transmission of infectious agents and also farmers indicated that tethering will prevent conflicts arising from destruction of crops in otherpeoples fields. It was observed that few farmers had knowledge on PPR in both border districts. Knowledge on how the PPR is transmitted can ensure timely reporting of the diseases to respective government for proper response. There was vaccination done in Tanzania side since 2012 and most farmers interviewed, 54.5% of the households in Kyela vaccinate their animals while in Ileje (65.2%)indicated to have not vaccinated their animals against PPR. Low vaccination coverage of PPR can ensure continued prevalence in areas where the disease has been reported to have occurred (Karimuribo*et al*., 2011). Most farmers indicated to consults veterinary officers in Chitipa (92.3%) and Ileje (87.0%) whilst in Kyela (97%) and Karonga (74.2%), farmers rely on information from field officers.Vaccination of small ruminants against PPR is the only measure that can stop spread of the disease by creating a buffer zone between the infected population and naive population.

CHAPTER SIX

6.0   CONCLUSION AND RECOMMENDATIONS

6.1   Conclusion

It can be concluded that:

1. Currently there is no active PPR infection along the border districts of Tanzania and Malawi.

2. Based on partial analysis, communal grazing and free roaming husbandry practices are potential factors for the spread of the disease in case an outbreak occurs.

3. Two animals were found to have low viral load in Chitipa using RT-PCR which indicates some presence of the virus.

6.2   Recommendations

1. Combination of appropriate strategies through vaccination against PPR virus, movement control, strict husbandry practices, prevention of contact with infected animals and pretesting and quarantine for specific period of introduced animals need to be designed and applied in order to prevent contact from infected animals.

2. The infected animals need to be followed further to determine the actual source from which it was brought to ensure close monitoring of the disease.

3. Further study be done to exhaustively cover on the risk factors that lead to the two animals found positive in a free PPR area.

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APPENDICES

Appendix 1: Check list used for factors leading to spreads of *peste des petits ruminants* (PPR) in goats and sheep along the Tanzania-Malawi border

Introduction

Peste des petits ruminants (PPR) has been confirmed in in several parts of Tanzania and these possess a great threat to the goats and sheep population to other parts which are not endemic to the disease. The disease is a trans-boundary animal disease A listed by the OIE as such Malawi is at a high risk. This research is part of the fulfillment of the requirements for the degree of Masters of Science in Epidemiology. It has been sponsored by the Africa Development Bank through SADC-TADs Project.

Date (dd/mm/yyyy): ………………… **Name of interviewer: ……………………**

Place of interview: ……………..…...Name of Respondent: …………………

Village………………………………… **Ward/EPA: ………………………**

District: …………………………… **Region: …………………………**

Gender of Respondent: **M** ☐ **F** ☐

Flock data

1. How many goats and sheep animals do you have in your farm and their categories?

   Goats: ☐ Bucks ☐ Does ☐ Weaners ☐ Kids ☐

   Sheep: ☐ Rams: ☐ Ewe ☐ Weaners ☐ Lambs ☐

Animal management data

2. Where do you take your animals for feeding?

   Communal grazing land   Tethering

Free roaming ☐

3. Do your animals mix with other peoples animals and share feed and water points?

Yes ☐   No ☐

4. Where do you sell your animals and how often?

Goat/sheep market ☐   Buyers who move around ☐

5. Have you introduced any animals from other farms for the past 6 months?

Yes ☐   No ☐

6. If the answer is yes in above question, how many?

Goats: ☐   Sheep ☐

Animal health data

7. Where do you get veterinary services when your animals get sick?

Veterinary Officer ☐   Field officer ☐

Do it myself ☐   Other People with animals ☐

8. Have you vaccinated your animal for the past 2 years?

Yes ☐   No ☐

9. Do you mix the young and old animals all the times?

Yes ☐   No ☐

10. What diseases have experienced for the past 1 year?

Helminth infestation ☐   *Petse des petits ruminants* ☐

Pneumonic Pasteurollosis ☐   Foot and mouth disease ☐

Contagious ecthyma ☐   Contagious CaprinePleuropneumonia ☐

Blue tongue ☐

11. What signs did you observed?

Respiratory distress ☐   Profuse Diarrhoea ☐

Coughing   Mucopurulent nasal discharges

Death of animal within a week ☐　　　Rise in body temperature ☐

Other signs observed ……………………………………………………

12. Who did you report to when the disease was noticed?

Veterinary Officer ☐　　　Field officer ☐

Other People with animals ☐

13. Do you know the disease called goat and sheep plague (*peste des petits ruminants*)?

Yes ☐　　　No ☐

14. How can you notice the disease?

………………………………………………………………………………………

15. If your goats/sheep are attacked by the disease above what measures do/can you take to ensure the other animals are safe?

Kill the animal ☐　　　Report to Vet officer ☐

Report to field officer ☐　　　look for Treatment ☐

Notify my neighbour's ☐　　　do nothing let it die ☐

16. Have you heard or seen this disease before in your area?

Yes ☐　No ☐

Appendix 2: c-ELISA test

The 96 well ELISA plates (CIRAD-EMVT, Montipllier, France) already coated with PPR recombinant nucleoprotein were used. All the reagents from the kit were first allowed to come to room temperature (21 ^{o}C) before use and all the reagents and sample sera were homogenized by vortexing. First of all, 25μl of dilution buffer 13 was added to each well followed by 25μl of positive control to wells A1 and B1 then 25μl negative control to wells C1 and D1. Afterwards, 25μl of each sample to be tested was added to the remaining 92 wells. The total serum dilution factor of 1:2 was attained as per instructions. The contents were then incubated at 37 ^{o}C for 45 minutes. Each well was then washed with 300μl of wash solution for three times and making sure that the wells do not dry of between washings. To each well, 100μl of conjugate 1X prepared from a 10X stock solution to 1/10 in dilution buffer 4 was added. The contents were then incubated at 21 ^{o}C for 30 minutes. Afterwards, each well was washed with 300μl of wash solution for three times and making sure that the wells do not dry of between washings. To each well, 100μl of substrate solution was added and then incubated at 21 ^{o}C for 15 minutes in a dark place. Lastly, 100μl of 0.5M stop solution was added to each well in order to stop the reaction. The optical density (OD) was read in an electrospectophotometer at 450nm and results recorded.

Assay performance and interpretation

The test validation was conducted as follows:

- ✓ The test is valid if the mean value of the negative control OD (OD_{NC}) is greater than 0.7 i.e. $OD_{NC} > 0.700$

- ✓ If the mean values of the positive control (OD_{PC}) is less than 30% of the OD_{NC}. i.e. $OD_{PC} / OD_{NC} > 0.3$.

For each sample, percentage inhibition (PI) or competition percentages were calculated using the formula shown below:

$$PI = 100 - \left[\frac{OD \text{ of each Sera}}{OD \text{ of Control}}\right] * 100$$

The test sera presenting a PI:

✓ Less than or equal to 50% were considered positive.

✓ Greater than 50% and less than or equal to 60% were considered doubtful.

✓ Greater than 60% were considered negative.

Appendix 3: Purification of viral RNA

In a 1.5 ml centrifuge tube, 150 µl of buffy coat, nasal swabs and 600 µl Lysis buffer with 2-mercaptoethanol were added. The contents were vortexed for 15 seconds until the cell pellets were dispersed and lysed. Another 150 µl of 70% ethanol was added to each tube then vortexed for 15 seconds to mix thoroughly and disperse any visible precipitates that may have formed after addition of ethanol. A total of 700 µl of the sample was transferred into a spin cartridge (QIAamp mini column) placed in a two ml collection tube. The column was centrifuged at 12,000x g for 15 seconds. The flow-through was discarded and the spin cartridge was reinstated into the same collection tube. If the solution has not completely passed through the membrane, centrifugation was repeated at higher speed until all the solution had passed through. The remaining final volume of the sample was transferred into the column, centrifuged at 12, 000 x g for 15 seconds after which the collection with filtrate was discarded and replaced with a clean two ml collection tube.

The bound nucleic acid was washed by adding 700 µl of Wash Buffer I to the spin cartridge and centrifuged at 12, 000 x g for 15 seconds at room temperature. The flow-through with the collection tube was discarded and the spin cartridge placed in a new clean collection tube. The second wash, 500 µl of Wash Buffer II with ethanol was added and centrifuged at 12, 000 x g for 15 seconds at room temperature. The flow-through was discarded and the step was repeated with Wash buffer II. The spin centrifuge was then centrifuge at 12, 000 x g for 2 minutes to dry the membrane with the bound RNA. The collection tube was discarded and the spin centrifuge placed in a recovery tube. A total of 60 µl RNase –free water was added to the centre of the spin cartridge and then incubated for 1 minute at room temperature. The contents were centrifuged at 17, 000 x g to elute the RNA from the membrane to the recovery tube. The filtrate, viral RNA was immediately stored at -20 ^{o}C until purification.

Appendix 4: Summary of practices and awareness by farmers with respect to PPR along the border districts of Malawi and Tanzania

Parameter	Chitipa		Karonga		Ileje		Kyela	
	freq	%	freq	%	freq	%	freq	%
Feeding practices								
Communal grazing	8	30.8	30	96.8	1	4.3	0	0.0
Leave to roam	1	3.8	0	0.0	1	4.3	17	51.5
Tethering	17	65.4	1	3.2	21	91.3	16	48.5
Total	**26**	**100**	**31**	**100**	**23**	**100**	**33**	**100**
Diseases experience								
no diseases	10	38.5	20	64.5	19	82.6	13	39.4
helminths	16	61.5	11	35.5	0	0.0	18	54.5
bacterial diseases	0	0.0	0	0.0	0	0.0	2	6.1
PPR like signs	0	0.0	0	0.0	4	17.4	0	0.0
Total	**26**	**100**	**31**	**100**	**23**	**100**	**33**	**100**
PPR awareness								
PPR knowledge								
yes	6	23.1	2	6.5	2	8.7	2	6.1
no	20	76.9	29	93.5	21	91.3	31	93.9
Total	**26**	**100**	**31**	**100**	**23**	**100**	**33**	**100**
PPR vaccination								
yes	0	0.0	0	0.0	8	34.8	18	54.5
no	26	100.0	31	100.0	15	65.2	15	45.5
Total	**26**	**100**	**31**	**100**	**23**	**100**	**33**	**100**
veterinary services								
veterinary officer	24	92.3	6	19.4	20	87.0	1	3.0
field officer	2	7.7	23	74.2	0	0.0	32	97.0
do it myself	0	0.0	0	0.0	3	13.0	0	0.0
other farmers	0	0.0	2	6.5	0	0.0	0	0.0
Total	**26**	**100**	**31**	**100**	**23**	**100**	**33**	**100**