-DISEASE STATUS AND RISK FACTORS FOR PESTE DES PETITS RUMINANTS ALONG TANZANIA-BURUNDI AND DEMOCRATIC REPUBLIC OF CONGO BORDER

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ABSTRACT

An epidemiological study was carried out between September 2011 and February 2012 to determine the disease status of Peste des Petits ruminants (PPR) in western Kigoma region bordering Burundi and Democratic Republic of Congo (DRC). The study aimed at establishing the seroprevalence of PPR and identifying risk factors associated with the disease in three districts (Kasulu, Kibondo and Kigoma rural) of Kigoma region. The study also assessed the presence or absence of clinical cases in the study area. A total of 35 sheep and 415 goats were sampled, 150 animals from each districts. Serological analysis employed monoclonal antibody based competitive Enzyme Linked Immunosorbent Assay (cELISA). A questionnaire survey was used to collect information on potential factors associated with the seroprevalence of the disease. In-depth interview of farmers with PPR seropositives and suspected cases was also conducted. The overall seroprevalence established was 5.1% (95% CI: 3.30%-7.70%). Stratification of the seroprevalence by district indicated that Kibondo had the highest seroprevalence (2.0%) followed by Kasulu (1.8%) and Kigoma rural (1.3%). Factors that had an impact on PPR spread includes communal grazing (p=0.01) and introduction of new animal in the flock (p=0.005). The potential risk factor associated with PPR seroprevalence and clinical cases was introducing new animal(s) in the flock from other locations in the study area (p=0.0054). To the best of my knowledge, this is the first study which has reported the prevalence of PPR in western region of Tanzania. As the study area borders neighbouring countries of Burundi and DRC, concerted efforts are required to jointly control the disease with the ultimate aim of eradicating it from the region.

DECLARATION

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

Signature:…… Date……..

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The above declaration is confirmed by

Signature:……. Date……..……..

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Descriptive meaning
%	Percentage
<	Less than
>	Greater than
≤	Less or equal to
≥	Greater or equal to
µ	Micro-10^{-6}
µl	Microlitre
^{0}C	Degree Celsius
AGID	Agar gel immunodiffusion
CCPP	Contagious caprine pleuropneumonia
CDC	Centre for Disease Control and Prevention
CDv	Canine distemper virus
cELISA	competitive Enzyme Linked Immunosorbent Assay
cH-ELISA	Competitive anti-H monoclonal based ELISA
CI	Confidence interval
CIEP	Counter immunoelectrophoresis
CVL	Central Veterinary Laboratory
DIVA	Differentiating infection in vaccinated animals
DMv	Dolphin morbilli virus
DNA	Deoxyribo nucleic acid
DRC	Democratic Republic of Congo
DVO	District Veterinary officer
EDI	ELISA Data Information software

ELISA	Enzyme-linked immunosorbent assay
EMPRES	Emergency Prevention System
F	Fusion protein
FAO	Food and Agriculture Organization
GIS	Geographic Information system
GPS	Global Positioning System
H	Hemaglutinin protein
ICE	Immunocapture ELISA
ILRI	International Livestock Research Institute
Km	Kilometre
M	Metre
MAb	Monoclonal antibody
Mm	Millimetre
MoLFD	Ministry of Livestock and Fisheries Development
Mv	Measles virus
OD	Optical density
OIE	Office Internationale des Epizooties (World Organisation for Animal Health)
OR	Odds ratio
PBS	Phosphate buffer solution
PCV	Packed cell volume
PDv	Porpoise distemper virus
pH	Hydrogen ion concentration
PI	Percentage inhibition
PMv	Phocine morbilli virus
PPR	Peste des Petits ruminants

PPRv	Peste des Petits ruminants virus
RBCs	Red blood cells
RPv	Rinderpest virus
RT-PCR	Real-time polymerase chain reaction
SADC	Southern African Development Community
SLAM	Signalling Lymphocyte activation Molecule
TADs	Trans-boundary Animal Diseases
UTM	Universal Transverse Mercator
WAD	West Africa dwarf

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Peste des Petits Ruminants (PPR) is an acute and highly contagious infectious viral disease of small domestic and wild stocks of high economic value. The disease is caused by *Peste des Petits Ruminants* virus (PPRv) which belongs to Morbillivirus genus of Paramyxoviridae family (Gibbs *et al*., 1979). The virus exist as a single serotype with four (I, II, III and IV) lineages based on the sequence comparison of a small region of the fusion (F) gene (Forsyth and Barrett, 1995) or nucleocapsid (N) gene (Couacy-Hymann *et al*., 2002). In Eastern Africa the only PPRv strain reported in the region is lineage III (Dhar *et al*., 2002; Khalafalla *et al.,* 2010).

The disease affects sheep and goats that constitute more than 30% of the domestic meat source in Africa (Reed *et al*., 1988) hence the present and future income generating ability is grossily affected (Ashley *et al.,* 2010). Besides, the disease impacts negatively the local and international livestock trade and may cause mortality rates of 50–80% in naive sheep and goats populations consequently affecting food security (Kitching, 1988). Generally, small ruminants are ready sources of food and cash for women and disadvantaged households and are important means of rebuilding herds after environmental and political shocks, especially in livestock-dependent communities. Peste des petits ruminants is likely to spread to most of Africa if not controlled thus bringing losses of livestock and endangering the livelihoods of millions of African livestock dependent communities (ILRI, 2012).

Peste des petits ruminants outbreak was first described and confirmed in Northern Tanzania in 2008 (Kivaria *et al.,* 2009; Swai *et al.,* 2009). As of 2010, the disease was

reported to threaten local population of over 13.5 million goats and over 3.5 million sheep in the country (FAO, 2010).

The disease has been suspected to spread to western and Southern highlands of Tanzania from Northern zone and neighbouring countries. Democratic Republic of Congo (DRC) declared the outbreak in 2005 (OIE, 2011). However, the exact epidemiology of the disease in the border areas is not well known. Scanty information available is from a sero-surveillance study conducted along Zambia - DRC and DRC-Tanzania border areas which reported sero-positive cases, although there was no clinical case observed (SADC, 2011). Other western countries bordering Tanzania (Burundi and Rwanda) have officially reported neither the presence of PPR clinical cases nor sero-conversions in their domestic or wild animal populations. However, uncontrolled movement of refugees from Burundi, DRC and Rwanda together with their livestock and trade of small ruminants for food and breeding in and out of Tanzania or along the borders have great potential to introduce and spread the disease in the Great Lake region.

Although there is no official PPR disease reports in Western and Southern Highlands zones of Tanzania, it is believed that this observation might be due to misdiagnosis due to weak surveillance, reporting and diagnosis systems (Karimuribo *et al.*, 2011a). In addition, the strains of PPRv circulating in the population have not been isolated and characterized. Such information could be useful towards improving our understanding in the epidemiology and control of this disease in Tanzania.

1.2 Problem statement and justification

There is growing appreciation that PPR is the most serious and escalating disease that affects livelihoods of the poorest farming families and as well as food security in the

East Africa region (FAO, 2010). Peste des petits ruminants was officially confirmed in northern Tanzania in 2008 (Kivaria *et al*., 2009; Swai *et al*., 2009). However, a retrospective study by Karimuribo *et al*. (2011b) reported at least seropositivity to PPR in samples collected in 1995. The disease spread southwards and in 2009, it had reached the southern part of Tanzania (Muse *et al*., 2012a). There has been a mounting concern of the disease due to its progressive devastating effects in goats and sheep and to the meat industry in Tanzania (MoLFD, unpublished report). Despite existing suspicions of PPR in most part of Tanzania, there is no information on the status of the disease in the western part of Tanzania. This study was designed to assess the status of PPR in the western Tanzania bordering DRC and Burundi in order to develop platform for setting control strategies against the disease.

1.3 Overall objective

1.3.1 Main objective

The overall objective of the study was to conduct an epidemiological investigation on PPR along the western border of Kigoma region neighbouring DRC and Burundi countries in order to assist devising the strategy to control and prevent the disease in this zone.

1.3.2 The specific objectives

i. To establish the seroprevalence and clinical status of PPR in goats and sheep in western Tanzania districts (Kigoma rural, Kasulu and Kibondo) districts bordering Burundi and DRC.
ii. To identify risk factors associated with the seroprevalence and disease spread along western Kigoma borders, Tanzania.

1.3.3 Hypothesis

Null hypothesis

There is no circulating PPRv in goat and sheep in western Kigoma villages along DRC and Burundi borders.

Alternative hypothesis

There is circulating PPRv in goats and sheep in western Kigoma villages along DRC and Burundi borders.

Research questions

i. Is there any PPRv circulating in goat and sheep in western Kigoma villages along DRC and Burundi borders?

ii. What are factors contributing to the introduction and spread of PPR in Kigoma region?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition of the disease

Peste des Petits ruminants is an acute highly contagious viral disease of domestic and wild ruminants caused by PPRv. The virus is an enveloped, non segmented negative strand RNA viruses within the family Paramyxoviridae (Gibbs *et al*., 1979). The PPR virus is antigenically and biologically related to rinderpest virus and clinically it mimics rinderpest in goats (Luka *et al*., 2011). The disease is clinically manifested by high fever, diarrhoea, oculo-nasal discharges, erosive stomatitis and crusting scabs along the lips, development of pneumonias in late stages and high mortality rates (EMPRES, 2009). The major sites of viral propagation are lymphoid tissues, and acute diseases are usually accompanied by profound lymphopenia and immunosuppression, leading to secondary and opportunistic infections (Appel and Summers, 1995; Murphy and Parks, 1999).The disease severity is influenced by several factors including farming system, flock movement, lack or inadequate disease control strategies and commitments by the government. Other factors include unavailability of resources to undertake surveillance and diagnostics procedures, PPRv lineage, animal species, breed, immune status and age whereas the case fatality rate in young animals is higher (Lefèvre and Diallo, 1990).

2.2 Aetiology

Peste des petits ruminants is caused by a virus which belongs to the *Morbillivirus* genus of the *Paramyxoviridae* family (Gibbs *et al*., 1979) called *Peste des petits ruminantis* virus (PPRv). The *Morbillivirus* genus also includes other six disease-causing viruses: rinderpest virus (RPv), measles virus (Mv), canine distemper virus (CDv), dolphin morbilli virus (DMv), porpoise distemper virus (PDv) and phocine morbilli virus (PMv) (Barrett *et al*., 1993b; Barrett, 2001).

Sequence comparisons of F-Protein genes were done for determination of genetic relationship between PPR viruses (Shaila *et al*., 1996; Dhar *et al.,* 2002). Four lineages of PPR virus were identified where lineage I and II viruses were exclusive reported in West Africa (Abraham *et al*. 2005). Peste des petits ruminants virus lineage IV is predominantly found in Middle East, Asian sub-continent, Nepal and Bangladesh. However, the geographical source of the lineage IV viruses is unknown although it is most closely related to African lineage I. It has also been reported in Turkey (Ozkul *et al*., 2002). Peste des petits ruminants virus of lineage III was reported in East Africa, Arabia and Southern India (Diallo, 1988) and Ethiopia (Roeder *et al.,* 1994).

2.3 Geographical distribution

2.3.1 Global situation

Peste des petits ruminants is endemic between the Sahara and the Equator in Africa, the Middle East and the Indian sub continent (Lefèvre and Diallo, 1990; Taylor *et al*., 1990; Roeder *et al*., 1994; Amjad *et al*., 1996). The disease has been endemic in West Africa and later spread across East Africa, the Middle East and Southern Asia as far as Bangladesh (Shaila *et al*., 1996) and Turkey (Ozkul *et al*., 2002). It is found in many African countries between the Atlantic Ocean and the Red Sea. It has been reported in Ivory coast (Gargadennec and Lalane, 1942), Senegal (Mornet *et al*., 1956), Chad (Provost *et al.*, 1972), Benin (Bourdin, 1973), Nigeria (Hamdy *et al.*, 1976., Taylor and Abegunde, 1979), Morocco (FAO, 2009), Sudan (El Hag and Taylor, 1988), Ethiopia (Roeder *et al.*, 1994 and Abraham *et al*, 2005), Kenya and Uganda (Wamwayi *et al.*, 1995). Outside Africa PPR has been reported in Saudi Arabia (Furley *et al.*, 1987 and Abu Elzein *et al*., 1990), India (Shaila *et al.*, 1989 and Nanda *et al*., 1996), Jordan (Lefèvre*et al.*,1991), Israel (OIE, 1993), Oman (Hedger *et al.*, 1980), and Pakistan (Amjad *et al.*, 1996). Marked rise in regional incidence of PPR outbreaks during recent

years Nanda *et al*., 1996; Shaila *et al*., 1996: Ozkul *et al*., 2002) indicates the trend of disease to spread. The presence of circulating virus was confirmed by serological determination in Syria, Niger, India, Turkey, Jordan and Pakistan whereas the virus presence was detected in Ethiopia and Eritrea (Roeder *et al*., 1994; Sumption *et al*., 1998; Abubakar *et al*., 2008) and in DRC (OIE, 2011).

2.3.2 PPR status in Tanzania

A retrospective study carried out showed that the disease was first suspected in Ngorongoro district back in 1995 (Kivaria *et al.* 2009, Karimuribo *et al.*, 2011a). However, Kivaria *et al*. (2009) officially confirmed widespread of PPR in Northern parts of Tanzania including Hai and Ngorongoro districts in 2008. Another study by Muse *et al*. (2012a) confirmed PPR cases in Southern part of Tanzania in Newala district in 2009 and Tandahimba districts in 2010. Other official reports of PPR were from Namtumbo in 2010, Ruvuma region in 2010, Masasi, Mtwara region in 2011, Kishapu and Meatu, Shinyanga region in 2011, Handeni Tanga region 2011 and Makuru Singida region in May 2012 (MoLFD,unpublished report).

2.4 Epidemiology

2.4.1 Transmission

Close contact between infected animals in the febrile stage and susceptible animals play a big role in transmission of PPRv (Braide, 1981). The transmission is mainly through the discharges from eyes, nose and mouth, as well as the loose faeces. Fine infective droplets are released into the air from these secretions and excretions, particularly when affected animals cough and sneeze (Taylor, 1984; Bundza *et al*., 1988). Animals in close contact with the affected individuals inhale the droplets and are likely to become infected. Although close contact is the most important way of transmitting the disease, it

is suspected that infectious materials can also contaminate water, feed troughs and bedding, turning them into additional sources of infection. Indirect transmission seems to be unlikely in view of the low resistance of the virus in the environment and its sensitivity to lipid solvent (Lefèvre and Diallo, 1990). It is also believed that there is no known carrier state for PPRv (Gopilo, 2005), although there are speculations of virus adaptability in other animal species without showing overt clinical signs. Peste des petits ruminants has now been demonstrated serologically in wild lion and clinically in camels (Khalafallaa *et al*., 2010). Trade in small ruminants, at live animal markets where animals from different sources are brought into close contact are some factors contributing to increased risk for PPR transmission, as does the development of intensive fattening units.

2.4.2 Host Range and susceptibility

Peste des petits ruminants is mainly a disease of small ruminants affecting goats and sheep. The disease virus exhibits different levels of virulence in sheep and goats. Goats are severely affected while sheep generally undergo a mild form of the disease (Lefèvre and Diallo, 1990). Infected sheep rarely suffer from clinical disease (El Hag and Taylor, 1988; Roeder *et al.,* 1994). Breed may affect the outcome of PPRv infection and its epidemiology. For instance, the Guinean breeds of goats (West African dwarf, Iogoon, kindi and Djallonke) are known to be highly susceptible (Lefèvre and Diallo, 1990). A more recent observation detected variations in breed susceptibility within goats in West Africa. For instance, the dwarf breeds of goats have been found to be more susceptible to PPR than the Sahelian breeds (Diop *et al.,* 2005; Couacy-Hymann *et al.,* 2007). Presence of other diseases and other stress factors precipitate the occurrence of the disease. Small wild ruminant species like antelope can also be severely affected by PPR (Abu Elzein *et al*., 2004). Other wild animals which can be affected by PPR include

gazelles (*Gazella dorcus*), ibex (*Capra ibex nubiana*), gemsbok (*Oryx gazelle*) and laristan sheep (*Ovis orientalis laristanica*) (Chauhan *et al*. (2009).

2.4.3 Morbidity

In goats, PPR has high morbidity especially in susceptible goat populations ranging from 80% -90% (Lefèvre and Diallo, 1990). Severe outbreaks can occur when naive animals are moved into an endemic area (Kusiluka and Kambarage, 1996). Infection rates in enzootic areas are generally high (above 50%) and can reach 90% during an outbreak (Radostits *et al.,* 2007). Young animals are generally very susceptible with kids being more severely affected than adult animals (Taylor *et al*., 1990).

Variable seasonal patterns of PPR occurrence have been reported. For instance, although outbreaks in West Africa generally coincide with the wet rainy season, Opasina and Putt (1985) observed outbreaks during the dry season in two different ecological zones. Taylor and Abegunde (1990) observed that incidence reflects an increase in number of susceptible young goats recruited into the flocks rather than seasonal upsurge in the virus activity, since its upsurge depend on the peak of kidding seasons.

2.4.4 Mortality

Mortality due to PPR has been reported to range between 50 and 80% (Lefèvre and Diallo, 1990). In arid and semi-arid regions, rarely PPR causes fatal effect although it may present itself as subclinical or inapparent infection (Lefèvre and Diallo, 1990). Case fatality rates are higher in goats (55-85%) than in sheep (less than 10%) (Abu-Elzein *et al.,* 1990).

2.4.5 Clinical signs

The acute form of PPR predominantly occurs in goats and rarely is reported in sheep. It is usually characterized by fast breathing, high fever (41^{o}C), depression, anorexia, purulent lacrimation, reddening of conjunctiva and mating of eyelids. Other signs include purulent nasal discharges, obstruction of the nose resulting into coughing and respiratory distress (Muse *et al.*, 2012b). Small nodular lesions in the skin on the outside of the lips around the muzzle and genital region have also been reported in later stages of the disease (FAO, 1999; Baron *et al.*, 2011; Muse *et al*., 2012b).

Foul-smelling material containing shreds of epithelial tissue usually occurs following gentle rubbing across the gum and palate with a finger (Braide, 1981; Muse *et al*., 2012a). Elevated PCV above 60% is common (normal 35-45%), with very high RBCs count and lymphocytopenia (Furley *et al*., 1987).

2.5 Pathology

Peste des petits ruminants virus is a lymphotropic and epitheliotropic like other morbilliviruses (Scott, 1981) thus induces severe lesions in organs rich in lymphoid and epithelial tissues. The portal of entry being the respiratory system, PPRv localizes first and replicate in the tonsil, mandibular and pharyngeal lymph nodes. After 2-3 days viraemia may develop following infection. The virus then disseminate to the spleen, bone marrow and mucosa of the gastro-intestinal tract and the respiratory system (Scott, 1981). Different post-mortem and histopathological lesions are associated with the PPRv infections.

2.5.1 Post mortem findings

Usually the carcass is emaciated while the eyeballs are sunken and the hindquarters are soiled with watery or soft faeces. On the nose and eyes, dried-up discharges are usually

seen, lips may be swollen, eroded and later nodules may be seen. Evidence of pneumonia is shown by dark red or purple lung with areas being firm to the touch on the anterior and cardiac lobes. Associated regional lymph nodes are also swollen and soft to touch, while haemorrhagic lesions are seen in the abomasum.

Erosion in the oral cavity is a constant feature in PPR while necrotizing and ulcerative lesions in the mouth and the gastro-intestinal tract is an important pathological findings (Roeder *et al*., 1994). In the small intestine, lesions are limited to small streaks of haemorrhages, erosions may occur in the first portion of the duodenum and the terminal ileum. More severe effect is seen in the large intestines around the ileo-cecal valve, ceco-colic junction and in the rectum where congestion is markedly seen. Discontinuous streaks of congestion characteristically observed as "zebra stripes" are usually seen on the posterior part of the colon and the rectum (Kusiluka and Kambarage, 1996).

Small erosion and petechiae haemorrhages may be seen on the nasal mucosa, turbinates, larynx and trachea. Bronchopneumonia, if present, is usually confined to the anteroventral areas, and is characterized by consolidation and atelectasis.

2.5.2 Histopathology

In the alimentary and respiratory tracts, PPRv causes necrosis of the mucosa epithelial lining marked by the presence of eosinophilic intracytoplasmic and intranuclear inclusion bodies. Brown *et al*. (1991) observed the presence of multinucleated giant cells in the affected lymph nodes and epithelial cells. The virus causes necrosis of lymphocytes in the spleen, tonsil and lymph nodes (Rowland *et al*., 1971). Immunohistochemical methods detected viral antigen in both cytoplasm and nuclei of tracheal, bronchial and bronchio-epithelial cell, type II pneumocytes, syncytial cells and alveolar macrophages (Brown *et al.*, 1991).

2.6 Immunity

Protective immunity of morbilliviruses is due to surface glycoproteins haemagglutinin (H) and fusion protein (F). Antigenically PPRv is closely related to rinderpest virus (RPv) and antibodies against PPRv are both cross-neutralizing and cross protective (Taylor and Abegunde, 1979). A double recombinant vaccinia virus expressing H and F glycoproteins of RPv protects goats against PPR disease (Jones *et al*., 1993). Capripox recombinants expressing the H protein or the F protein of RPv or the F protein of PPRv conferred protection against PPR disease in goats, but without production of PPRv-neutralizing antibodies (Romero *et al*., 1995).

Sheep and goats are unlikely to be infected more than once in their life time (Taylor, 1984). Lambs or kids receiving colostrum from previously exposed or vaccinated animals with rinderpest tissue culture vaccine were found to acquire a high level of maternal antibodies that persist for 3-4 months. The maternal antibodies were detectable up to 4 months using virus neutralization test compared to 3 month with competitive ELISA (Libeau *et al*., 1992). Small ruminants could only be protected against PPR by using homologous attenuated vaccine (Couacy-Hymann *et al*., 1995).

2.7 Diagnosis

Laboratory analysis is important in diagnosis of PPR by performing various laboratory based tests including virus isolation, detection of viral antigens, and nucleic acid sequencing and detection of specific antibody in serum.

2.7.1 Virus isolation

Samples from live goats and sheep that could be used for virus isolation include nasal, eye swabs and heparinized blood. Samples from dead animals that may be used for

virus isolation include mesenteric lymph nodes, tonsil, spleen, lungs and colon section. Collection of samples must be done during the viraemic phase and transported on cold ice for testing in laboratory (Lefèvre, 1987). Virus isolation in cell culture can be attempted with several different cell lines, although recovery of virus is not always successful. Previous studies recommended to use marmoset-derived cell line (B95a) (Screenivasa *et al.,* 2006), primary lamb kidney or African green monkey kidney (Vero) cell cultures (Mahapatra *et al*., 2006). However, morbilliviruses are now recovered and grown in Vero cells (Seki *et al.,* 2003). Generally, cultures are examined for cytopathic effects following infection of a monolayer with suspect material; the identity of the virus can be confirmed by virus neutralization or molecular techniques (Singh *et al*., 2009).

2.7.2 Serology

Detection of antibodies to PPRv is generally carried out using ELISA techniques. Currently, the OIE recommends use of the competitive PPRv specific anti-H monoclonal based ELISA (cH-ELISA) (Anderson and McKay, 1994) and virus neutralization tests (FAO, 1996). However, several alternative tests do exist (Libeau *et al*., 1995; Choi *et al*., 2005) including the use of indirect Nucleocapsid ELISA (Ismail *et al*., 1995), immunofiltration (Dhinakar *et al*., 2008), a novel sandwich ELISA (Saravanan *et al*., 2008), haemagglutination tests (Dhinakar *et al*., 2000; Ezeibe *et al*., 2008) and latex agglutination tests (Keerti *et al*., 2009).

Detection of PPRv antigens can be performed using a variety of tools including immunocapture ELISA (ICE); (Libeau *et al*., 1994), counter immunoelectrophoresis (CIEP) or agar gel immunodiffusion (AGID) (FAO, 1996). The CIEP and ICE can distinguish PPRv from RPv, but the AGID test cannot differentiate these two viruses.

Agar gel immunodiffusion is also relatively insensitive, and may not be able to detect small quantities of viral antigens in milder forms of PPRv. Immunofluorescence and immunochemistry can also be used on conjunctival smears and tissue samples collected at necropsy.

2.7.3 Molecular diagnosis

Molecular techniques require sensitive and specific detection methods, such as standard Reverse transcription polymerase chain reaction (RT-PCR) (Forsyth and Barrett, 1995; Couacy- Hymann *et al.*,2002) and currently real-time PCR assays specific for PPRv (Bao *et al*., 2008; Kwiatek *et al*., 2010) and loop-mediated isothermal amplification techniques (Wei *et al*., 2009) have been used. The generation of a standard RT-PCR product is, however, necessary in order to perform sequence analysis and subsequent phylogenetic characterization of novel virus isolates. Extensive validation of these diagnostic techniques is required before they can be accepted as approved OIE diagnostic methods.

2.7.4 Differential diagnosis

The differential diagnosis of PPR includes rinderpest, contagious ecthyma, goat/sheep pox, Nairobi sheep disease, blue tongue, Contagious Caprine Pleuro Pneumonia (CCPP), pneumonic pasteurellosis, salmonellosis, colibacillosis and parasitic gastro-enteritis. Rinderpest is differentiated from PPR by serum neutralisation test. Contagious ecthyma, unlike PPR, is not associated with intestinal lesions unless there is secondary bacterial infection. Nairobi sheep disease is not severe in goats and no oral lesions are observed. Furthermore, Nairobi sheep disease is restricted to areas where the vector tick, *Rhipicephalus appendiculatus* is found. Bluetongue can be differentiated from PPR by close examination of the feet lesions and diarrhoea is not a feature of Blue tongue

disease. Contagious Caprine Preuropneumonia is primarily a disease of the respiratory system affecting goats and unlike in PPR, no mucosa lesions or diarrhea are observed in uncomplicated cases. Pneumonic pasteurellosis, salmonellosis and colibacillosis can be differentiated by isolation of the causative bacteria while in parasitic gastro-enteritis, demonstration of high egg counts or worm burdens differentiate it from PPR (Kusiluka and Kambarage, 1996).

2.8 Control

Small ruminant population structures differ significant from that of cattle but yet the control strategies for PPRv are similar to those used in eradication of RPv (Barrett *et al*., 1993b). Treatment against the PPR disease is not available, but control against the disease in non-endemic areas may be achieved using different measures such as quarantine, test and slaughter and proper disposal of carcasses, restriction of importation of sheep and goats from affected areas. In case of suspected cases of introduction, contact fomites and decontamination of affected premises is very important for the control of the disease in non-endemic areas.

Control of PPR in endemic areas is done through adoption of vaccination programmes using live attenuated PPRv vaccines such as Sungri 96, Arasur 87 and Coimbatore 97 (used in India) (Saravanan *et al.*, 2010) and Nigeria 75/1 strain (Diallo, 2003). Immunization of small ruminants with lymph node and spleen materials containing virulent virus inactivated with 1.5-5% chloroform was tried and the animals were immune to subsequent challenge 18 months later (Braide, 1981). Live attenuated rinderpest vaccine was also used to protect small ruminants against PPR but this practice was stopped to avoid false positive detection of RPv during the final stages of the rinderpest eradication campaign in order to enable a country to seek OIE recognition

for freedom from the disease (FAO, 2007). Regardless of the level of viral presence within an area, sero-monitoring through surveillance initiatives remains a critical tool in combating PPRv infection and preventing further spread.

Multivalent vaccines are currently being developed that may both protect vaccinated animals against several viral pathogens and enable vaccinated and infected animals to be distinguished using differentiating infected from vaccinated animals (DIVA) tests. Currently, vaccines exist based on the incorporation of PPRv immunogens into vectors such as sheep and goat pox (Diallo *et al*., 2002; Berhe *et al*., 2003; Chaudhary *et al*., 2009; Chen *et al*. 2010). Attempts are also being made to develop new vaccines based on recombinant DNA technology (Diallo *et al*., 2007).The cost of vaccines and their administration and the nature of sheep and goat production system make regional vaccination campaigns problematic and a worldwide vaccination campaign for PPRv unlikely.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted in Kasulu, Kibondo and Kigoma rural districts in Kigoma region along Burundi and DRC borders (Figure 1). This area lies between latitudes 3.6º and 6.5º south of Equator and longitudes 29.5º and 31.5º east of Greenwich along the shores of Lake Tanganyika. The study area borders Burundi and DRC in the west with approximately 50 km width strip along the border. Kigoma region has a tropical climate with Lake Tanganyika influencing the climate leading to high temperature and humidity. The average temperature ranges between 20 ºC and 30ºC. The annual total rainfall ranges between 600 and 1,600 mm, mostly distributed along and around the Lake Tanganyika and the highlands of Kibondo and Kasulu Districts (MPEE, 2008). The mean annual rainfall is about 1,100 mm starting from October and continues up to May, followed by a prolonged dry season. Kigoma region's land surface is hilly, ranging between 800 and 2,400 m above sea level. Of the three districts involved in this study, Kibondo is the largest followed by Kigoma rural and Kasulu occupying 35.6 %, 25.2% and 20.2% of the total area of Kigoma region, respectively (MPEE, 2008).

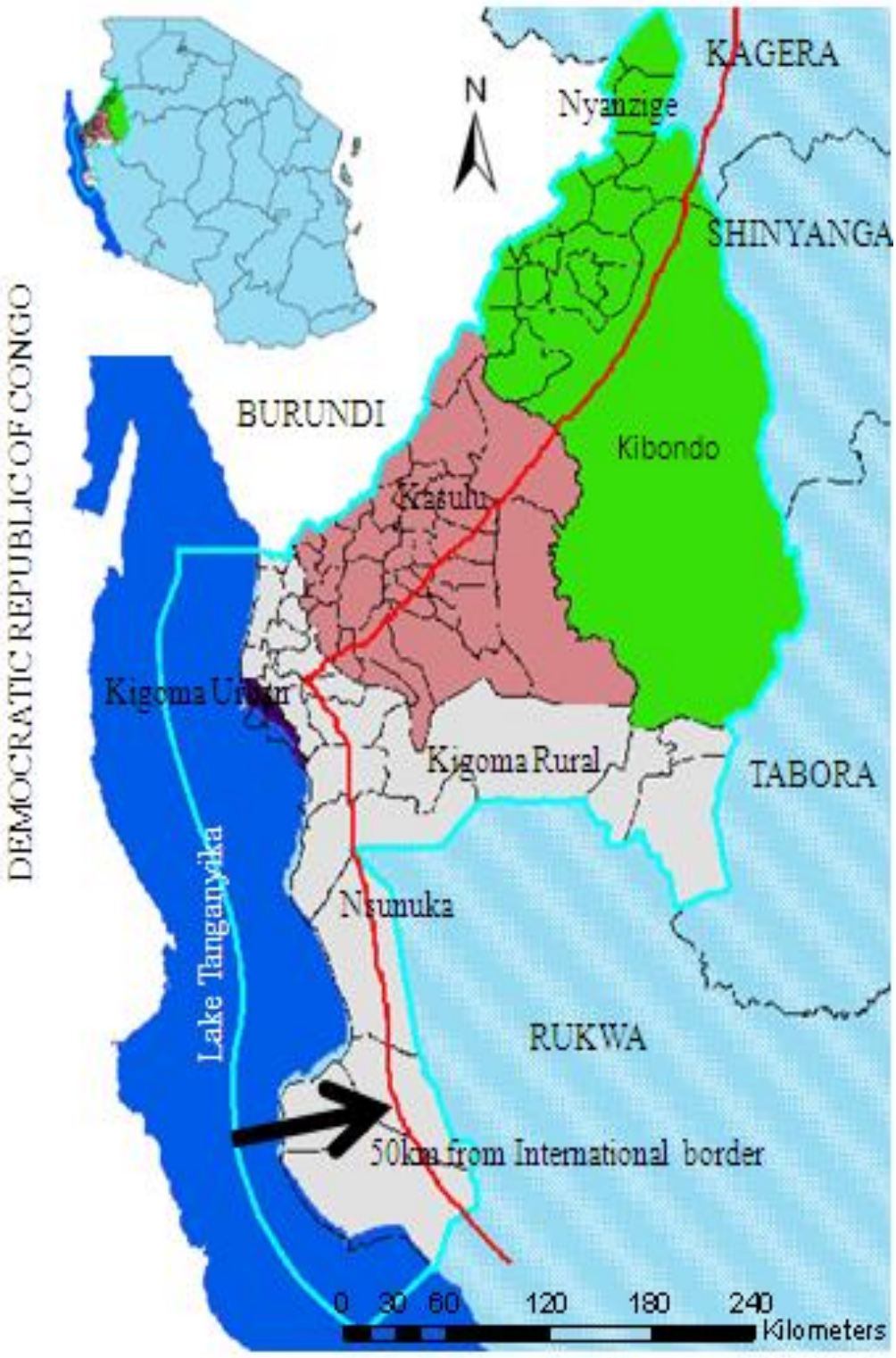

Figure 1: Map showing study area, a 50 km strip along the Tanzania-Burundi and Tanzania-DRC borders

3.2 Type of small ruminants reared in the study area

Animals included in the study were indigenous and crosses of Small East African and Red Maasai goats and Blackhead Persian sheep. These animals are found locally in the study area, some were either born and reared in the study area or had been moved in either through buying or brought in from another area nearby or even from the neighbouring countries like Burundi and DRC. The distribution of goats and sheep in the three study districts is summarized in Table 1.

Table 1: Small ruminant statistics in the study area stratified by district

Species	Kigoma rural	No/**HH	Kasulu	No/**HH	Kibondo	No/**HH	Animal Total
*Goats	198 238	24 779	65 935	7 326	94 366	8 579	358 539
*Sheep	24 143	4 828	9 107	2 276	11 212	2 803	44 462

* Source: District Agriculture and Livestock Offices in Kigoma rural, Kasulu and Kibondo.

**HH: House holds

3.3 Management system of small ruminant in the study area and possible interactions with neighbouring countries

Goat and sheep production in Kigoma region is mainly characterized by extensive and semi-intensive management, which includes sedentary and transhumance husbandry systems. Extensive type mostly depends on availability of natural grasslands during the rainy season. In some few flocks, animals are also supplemented with crop residues during the dry season while the majority do move away to look for pastures and water and this serves as a possible source of small ruminant interaction with other animals from neighbouring countries especially during the dry period when there is limited availability of pasture. Sedentary farming system is a feature of the agro-pastoralists mostly the indigenous Waha people while transhumance prevails in the pastoralists (the

Sukuma) mostly invading the forest and refugees mostly migrating from neighbouring Burundi seeking domicile in Kigoma region. The majority of households in the study area are sedentary farmers with less than ten animals (goats and sheep) while pastoralists usually keep more than ten animals and mostly found on the Eastern part away from the border of the study area neighbouring Tabora and Shinyanga regions.

3.4 Selection of study flocks

Households with ≥10 goats and / or sheep were included in the study by random selection from the sampling frame. Initially, purposive selection was carried out targeted district villages within 50 km strip from Tanzania-DRC and Tanzania and Burundi borders. In case the number kept was less than 10, nearby household goats and/or sheep were included to make ≥10 animals and that was grouped as one flock. The sampling frame was established from a list of all households keeping ≥10 goat and/ or sheep available at the village executive office. Small ruminant flocks were used as sampling units. Thus, the study evaluated 15 flocks from each of the three districts; hence 45 border villages found along the strip in the three districts were randomly selected and participated in the study.

3.5 Study design

A cross-sectional epidemiological study was adopted and two field visits were carried out between September 2011 and February 2012. The first visit was done from September to October 2011 which involved examination and sampling of goats and sheep along the Tanzania-Burundi border between Nyanzige village in the north and Nsunuka in the South (Figure 1). Questionnaire survey was also used to record information as described in section 3.7. The second visit was carried from January to February 2012 and adopted a case-control design. Cases in second visit were

farms/flocks with seropositive animals during the first visit and controls were flocks with negative animals which were matched to the positive flocks. The questionnaire survey was used and detailed interviewing of key respondents from households with seropositive recorded after analysing samples collected during the first visit matched with negative flocks was done. Clinical cases and potential risk factors that might have been the source of the seropositivity and clinical cases in the study area were recorded.

Sample size

Sample size was determined using a method recommended for 2-stage cluster sampling (Bennett *et al*. 1991; Thrusfield, 1995).

Sample size calculation

The sample size was calculated as

$n=Z^2PQ/L^2$ ..... (1)

Where n= sample size

Z=z value for a given confidence level

P= prevalence estimated

Q= (1-p)

L=allowable error of estimation

Since no known prevalence P =50% to give the maximum sample size

$n= (1.96^2* 0.5*0.5/0.05^2=384$

SE (standard error) = $\sqrt{}$ {p (1-p) D/n} where D= Design effect..... (2)

D= design effect given by = 1+ (b-1) *roh*..... (3)

Where *roh* = rate of homogeneity ≈ 0.2

b= average number of sample per cluster =10

D = 1+ (10-1) * 0.2

D = 1+ (0.9 * 0.2)

D = 2.8

SE = 0.043

Estimating sample size for cluster is given by the formula (Bennett *et al*., 1991)

$$C=p\,(1-p)\,D/s^2\,b \quad \text{..... (4)}$$

$$C= 37.85\approx 38$$

The numbers of clusters (villages) were increased from 38 to 45 for precision.

3.6 Blood sampling

Blood samples (415 from goats and 35 from sheep) were collected from the jugular vein and then allowed to clot at room temperature. The number of sheep sampled was lower as compared to that of goats because of fewer sheep kept by farmers in the study area. The sera were separated by decantation and transferred into 1.5 ml cryovials, labelled and transported chilled to the Central Veterinary laboratory for storage (CVL) where they were preserved at −20°C until analysis carried out.

3.7 Questionnaire Survey

A structured questionnaire administered during the first visit (September 2011 to October 2011) was used to collect information on location (identified by using Global Positioning System) of flock, flock size, animal species, health status, vaccination history and any clinical information observed by the farmers. The GPS coordinate of the sampled flock was recorded using Universal Transverse Mercator (UTM) system using GPS MAP device 60 CS, Garmin® Asia corporation No 68 Janshu 2nd Road, Shijr Taipae County, Taiwan.

The questionnaire survey carried out during the second visit between January and February 2012, targeted collection of data from key informants owners of flocks that

had at least one animal tested positive for PPR in the laboratory (n=23) as well as from none seropositive neighbours control flocks (n=42) matched with the positive flocks in each of the 3 districts. The questionnaires contained information on flock size, sex and age category classified as sub adult (<4 month-1year) and adult (>1year). Additional data collected were on health status based on history of abortion and history of previous PPR disease. Flock management data were also collected and included grazing system, source of drinking water, availability of animal health services, vaccination against any disease, contact with other animals particularly cattle, frequent visiting market with goats and/ or sheep and introduction of new animal(s) into study flocks.

3.8 Clinical examination of sick goats

Clinical examination of affected animals in Kibondo district was carried out. The examination involved observation of animals at a distance for general body condition, posture, respiration and general body movement. Close examination was also conducted to document/ record presence of lesion observed throughout the body at the head, nostril, muzzle, eyelids, genital organs and the skin regions.

3.9 Laboratory analysis of samples

3.9.1 cELISA analysis

A monoclonal antibody (MAb) based competitive Enzyme Linked Immunosorbent Assay (cELISA) was used for the detection of antibodies in sera as described by Singh *et al.* (2004a). The test depends on inhibition of binding of a mouse MAb to a PPR-specific epitope in the presence of positive serum. The test was conducted on a dust free bench at room temperature.

Briefly, the procedure involved the following steps;

1) 50 µl of PPRv antigen solution (working solution) diluted in coating buffer (PBS, pH 7.4) were added to all wells of the plate and tapped on the side of the microplates to ensure that the antigen evenly distributed over the bottom of each well and incubated for an hour at 37°C on an orbital shaker.
2) Plates were washed three times in washing buffer and blot dried.
3) Blocking buffer of 45 µl was added to all wells, then further addition of:

- 5 µl of blocking buffer to the monoclonal control wells (F1, F2, G1 and G2).
- 55 µl of blocking buffer to the conjugate control wells (A1 and A2).
- 5 µl of test serum to test wells.
- 5 µl of strong positive control to control wells (B1, B2, C1 and C2).
- 5 µl of weak positive serum to control wells (D1, D2, E1 and E2).
- 5 µl of negative serum to control wells (H1 and H2).
- 50 µl of monoclonal antibody diluted 1/100 in blocking buffer to all wells of the plate except the conjugate control (A1 and A2), followed by incubation for 1 hour at 37°C on orbital shaker.

4) The plates were washed three times and blot dried.
5) Addition of 50 µl of anti-mouse conjugate diluted 1/1000 in blocking buffer was done followed by incubation for 1 hour at 37°C.
6) Plates were washed three times.
7) Addition of 50 µl of substrate/chromogen solution was done in each well and colour development was stopped after 10 minutes by adding 50 µl of 1M sulphuric acid. The ELISA reader (BioTek®) connected to a computer loaded with ELISA Data Information (EDI) software was used at 492nm. A blanking plate was used (i.e. column 1 filled with substrate and stopping solution).

8) The inhibition of monoclonal antibodies (mAb) binding in the presence of serum was expressed as percentage inhibition (PI), calculated from mean optical density (OD) values using the formula:

PI = 100 - [(OD of the test wells/ OD of the Cm wells) x 100]

Sera showing PI equals to or greater than 50% were considered to be PPR positive.

3.10 Data analysis

Data were entered, screened for errors and stored in Microsoft Excel 2007 (version 12) and analysis was done by using Epi Info™ 7. 0. 9.7 software (CDC, 2012). The descriptive statistics were computed including the prevalence which was calculated as the proportion that tested positive in all samples tested. The multivariate logistic regression model was used to assess the association between the potential risk factors associated with the outcome variable defined by the seroprevalence of PPR. The level of significance in the univariate logistic regression analysis was p-value $\leq$ 0.3. A risk factor with P-value $\leq$ 0.05 was considered to be significantly associated with PPR in the multivariate model. Geo-spatial analysis of collected data was carried out using an Arc View® GIS (GeoInformation International, 1997).

CHAPTER FOUR

4.0 RESULTS

4.1 Animal and flock information

This study included 45 flocks with 450 goats and sheep in which 92.2% and 7.8% were goats and sheep, respectively (Table 2). Among these animals, males were 24.2% while females were 75.8%. The animals were categorized into two age groups namely adult and sub-adults that constituted 84% and 16% of total animals respectively.

The proportion of goat to sheep sampled in the three districts was higher with Kibondo having more goat sampled (99.7%) and Kigoma rural with less proportional of 82.7%. The percentage of sheep sampled in Kigoma rural was higher (17.3%) and Kibondo having less 0.7% than all districts (Table 2).

Table 2: Characteristics of animals and flocks sampled in Kasulu, Kibondo and Kigoma Rural districts, Tanzania.

Variable	Level	Kasulu (n=150) No (%)	Kibondo (n=150) No (%)	Kigoma(R) (n=150) No (%)	Total (n=450) No
Species	Goat	142 (94.7)	149 (99.3)	124 (82.7)	415
	Sheep	8 (5.3)	1 (0.7)	26 (17.3)	35
Sex	Female	122(81.3)	114 (76)	105 (70)	341
	Male	28 (18.7)	36 (24)	45 (30)	109
Female	Goat	85(56.7)	117 (78)	114 (76)	316
	Sheep	20(13.3)	5 (3.3)	0 (0)	25
Male	Goat	39(26)	25 (16.7)	36 (24)	100
	Sheep	6(4)	3 (2)	0 (0)	9
Age category	Adult	112(74.7)	132 (88)	134 (89.3)	378
	Su-adult	38(25.3)	18 (12)	16 (10.7)	72
Adult	Goat	108 (72)	112 (74.7)	132 (88)	352
	Sheep	26 (17.3)	0 (0)	0 (0)	26
Sub-adult	Goat	16 (10.7)	38 (25.3)	18 (12)	72
	Sheep	0 (0)	0 (0)	0 (0)	0
*Flock size	<10	46 (30.7)	65 (43.3)	65 (43.3)	176
	10-100	104 (69.3)	85 (56.7)	85 (56.7)	274
** <10	Goat	9 (6)	46 (30.7)	65 (43.3)	120
	Sheep	6 (4)	0 (0)	0 (0)	6
**10-100	Goat	115 (76.7)	104 (69.3)	85 (56.7)	304
	Sheep	20 (13.3)	0 (0)	0 (0)	20

* means number of flocks with goats and sheep together
** means number of flocks with goats or sheep

4.2 PPR serostatus in the study area

The flock level seroprevalence sampled was defined as the presence of at least one seropositive goat or sheep in the flock. Distribution of animals that were found positive to PPR is shown in Table 3. The results indicated that in goats, Kibondo had the highest seroprevalence and Kigoma rural recorded lowest seroprevalence while for sheep Kigoma rural had the highest seroprevalence and no seropositive sheep was detected in the Kibondo and Kasulu.

Table 3: Seroprevalence of PPR for goat and sheep sampled in Kasulu, Kibondo and Kgoma rural districts, Tanzania.

Variable		Kasulu (n=150)			Kibondo (n=150)			Kigoma(R)(n=150)			Overall % (n=450)
c-ELISA	Level	+ve	n	%	+ve	n	%	+ve	N	%	450
Species	Goat	8	142	5.6	9	149	6.0	3	124	2.4	4.4
	Sheep	0	8	0.0	0	1	0.0	3	26	11.5	0.7
Sex	Male	1	28	3.6	3	36	8.3	3	45	6.7	1.6
	Female	7	122	5.7	6	114	5.3	3	105	2.9	3.5
Male	Goat	1	25	4.0	3	36	8.3	0	39	0.0	0.9
	Sheep	0	3	0.0	0	0	0.0	0	6	0.0	0
Female	Goats	7	117	6.0	6	113	5.3	3	85	3.5	3.6
	Sheep	0	5	0.0	0	1	0.0	3	20	15.0	0.6
Age category	Adults	7	112	6.3	7	132	5.3	5	134	3.7	4.2
	Sub adults	1	38	2.6	2	18	11.1	1	16	6.3	0.9
Adults	Goats	7	109	6.4	7	132	5.3	2	108	1.9	3.6
	Sheep	0	3	0.0	0	0	0.0	3	26	11.5	0.7
Sub adults	Goat	1	38	2.6	2	18	11.1	1	16	6.3	0.9
	Sheep	0	0	0.0	0	0	0.0	0	0	0.0	0.0
*Flock size	<10	3	46	6.5	6	65	9.2	5	15	33.3	3.1
	10-100	5	104	4.8	3	85	3.5	1	135	0.7	2.0
**<10 flock size	Goats	5	41	12.2	6	64	9.4	3	9	33.3	3.1
**<10 flock size	Sheep	0	5	0.0	0	1	0.0	2	6	33.3	0.4
**10-100 flock size	Goats	3	101	3.0	3	85	3.5	0	115	0.0	1.3
**10-100 flock size	Sheep	0	3	0.0	0	0	0.0	1	20	5.0	0.3

* means number of goats and sheep together in sampled flock
** means number of goats or sheep kept in sampled flock
+ve means seropositive for c-ELISA

The seropositivity of PPR by village and district is shown in Figure 2. A village was considered positive if there was at least one seropositive animal in the cluster. Kibondo district had the highest prevalence of 2.0% while Kigoma rural had the lowest seroprevalence. Village-wise, Kalinzi (Kigoma rural) had the highest seroprevalence compared to other villages followed by Kagezi, Buhigwe, and Karumo.Least seroprevalence was recorded in Kibuye, Kumsenga, Nyanzige, Rumashi, Kalege and Kibwigwa villages.

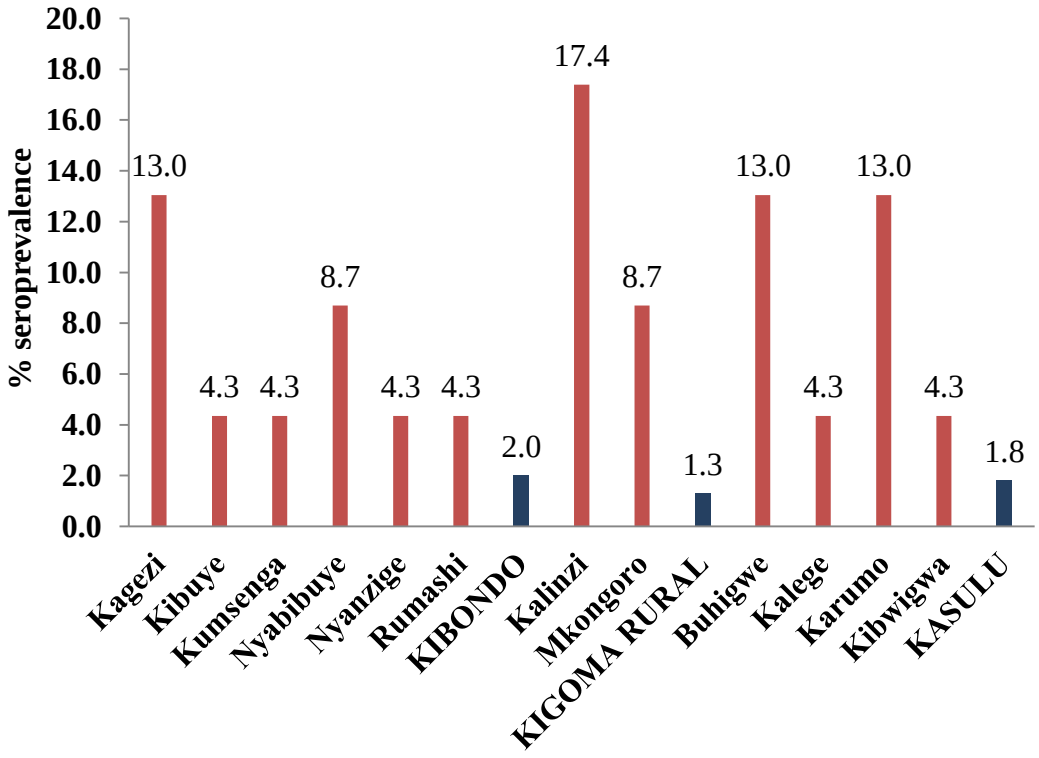

Figure 2: Seroprevalence of PPR in different villages (Red bar) and by Districts (black bar) (Capitals) (n=23)

Spatial distribution shows that PPR was prevalent from Nyanzige village (Kibondo) in the northern to Kalinzi (Kigoma rural) in the southern area. It was also observed that villages that recorded the highest seroprevalence in Kigoma Rural (Kalinzi) and Kasulu (Buhigwe and Karumo) districts were neighbours. Furthermore it was observed that a

new secondary livestock market at Buhigwe was located near the Tanzania-Burundi border which was likely to receive animals from neighbouring country of Burundi.

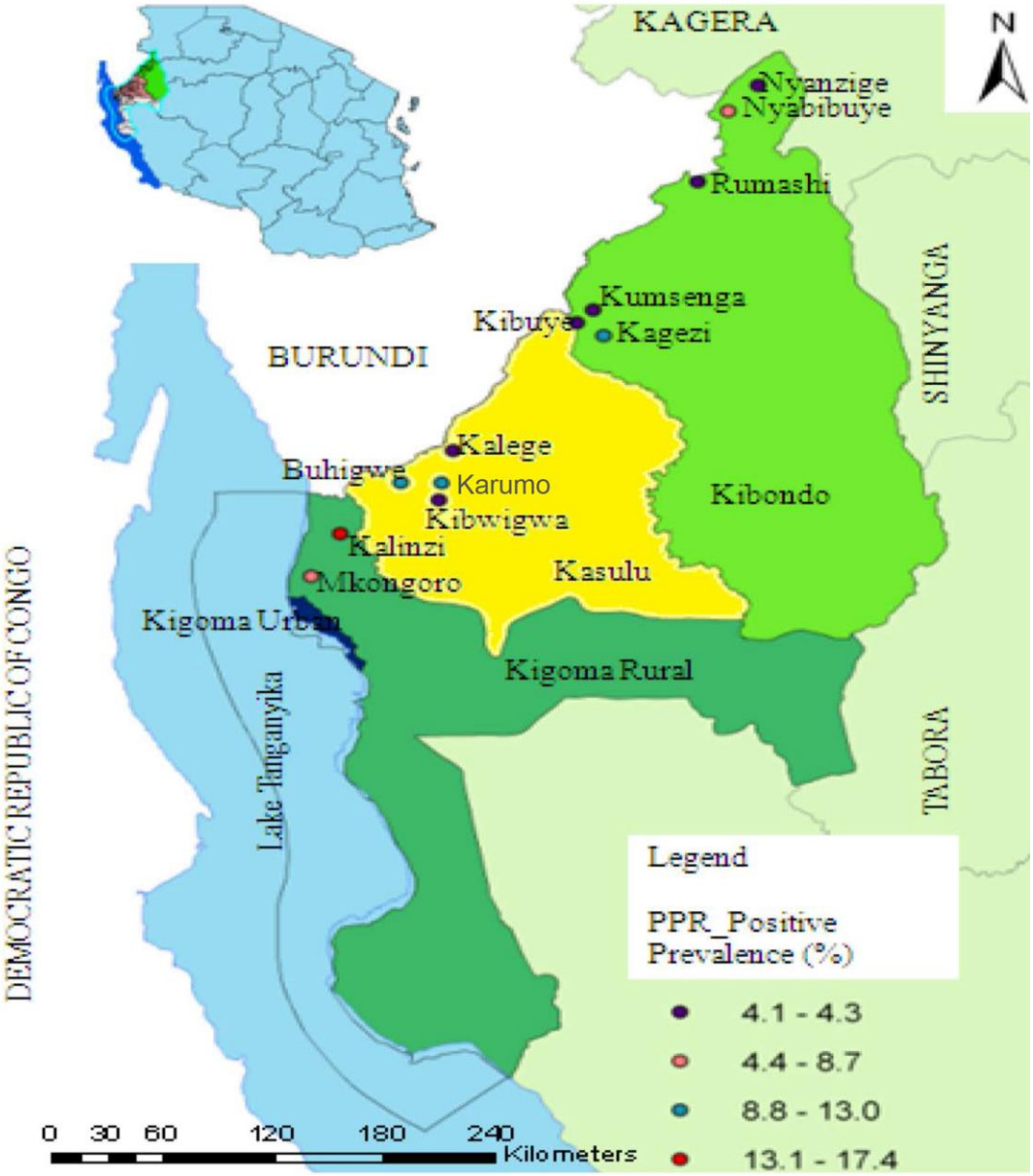

Figure 3: Showing spatial distribution of PPR in Kigoma region, Tanzania (n=23)

During the disease outbreak which occurred only in Kibondo, most famers responded by reporting the new disease to the nearby local field officer while few famers sold the affected animals to meat vendors. Other actions taken by farmers showing different response against the disease outbreak in Kibondo are shown in Figure 4.

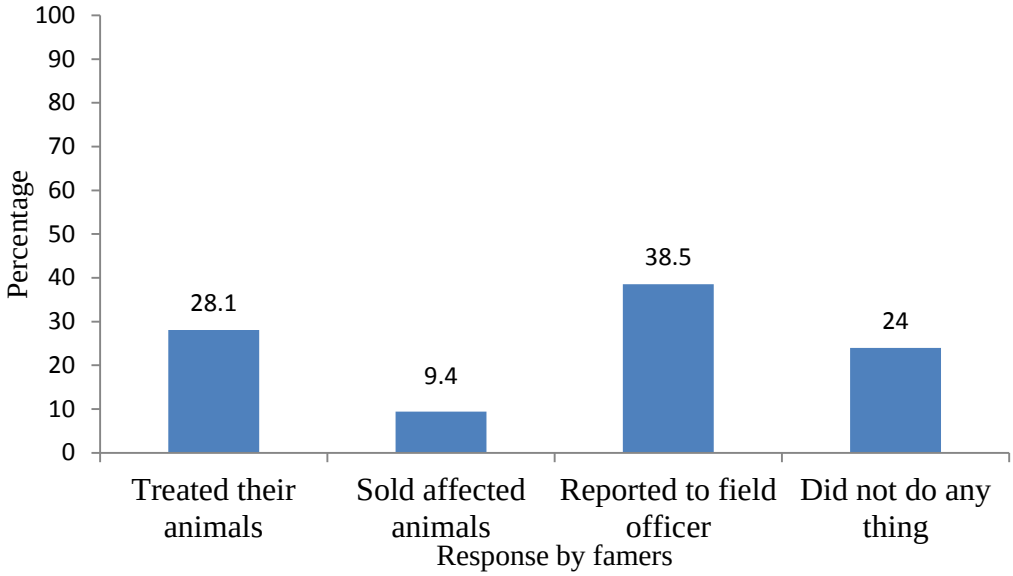

Figure 4: Showing the action taken by farmers on PPR infected animals in Kibondo district Kigoma, Tanzania (n=32)

Goats and sheep were housed in burnt brick houses thatched with grasses in most households. The majority of animal houses had earthed floors with very small windows and poor ventilation (Figure 5). Few household had wooden raised flows thatched with glasses or old iron sheets

Figure 5: Showing animal housing in Kibondo district Kigoma, Tanzania

Univariate logistic regression analysis revealed three variables as risk factor for PPR in sheep and goats in the study area. The three potential factors that qualified to be included in the multivariate model included ventilation in animal house, introduction of new animal in the flock and grazing system practised in the study area (Table 4a).

Factors that were not associated with PPR serostatus includes herd size (p= 0.5), age (p=0.4), contact with cattle (p=0.8), species affected (p=0.9), watering points (p=0.7) and floor type (0.5).

Table 4a: Results of the univariate associations with seropositive status against PPR Kibondo district Kigoma, Tanzania.

Risk factor	OR	95%	C.I	S.E	p-value
House ventilation , (Good /poor)	0.3676	0.0624	2.1674	0.9052	0.269
Introduced new animal in the flock, (Yes/No)	6.9333	1.7726	27.1187	0.6959	0.005
Grazing system, (tethering / communal grazing)	0.2715	0.0961	0.7668	0.5295	0.0138

Table 4b: Results of risk factor of introducing a new animal that was associated with PPR sero-positive status Kibondo district Kigoma, Tanzania.

Risk factor	OR	95%	C.I	S.E	P-value
New animal (Yes/No)	6.933	1.7726	27.1187	0.6957	0.0054

From the potential risk factor identified during the univariate analyses, only one factor was found to be statistically significant associated with increased odds of being cELISA positive. This factor was introduction of new animals in the flock from elsewhere (p value= 0.0054) (Table 4b).

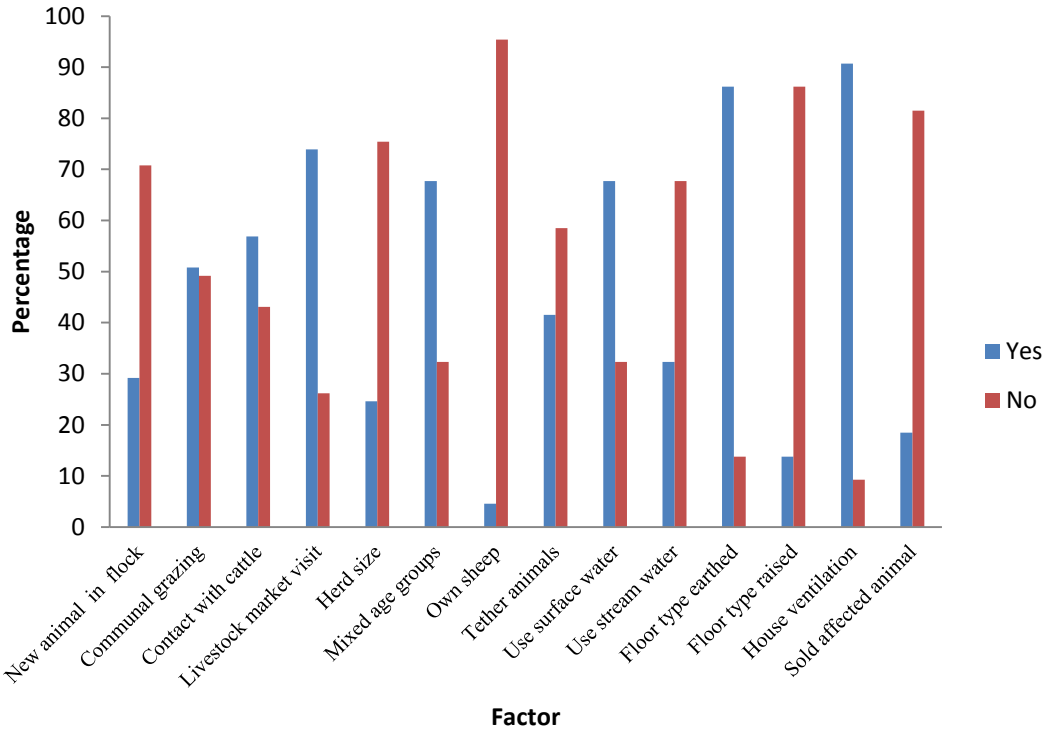

Figure 6: Distribution of responses of interviewees who had seropositive cases in their flocks

Among the grazing systems used in all districts, tethering was common while fewer respondents said to own sheep (Figure 6). Most of respondents had animal houses with good ventilation with ground earthed floor (Figure 6).

4.3 Clinical status of PPR in the study area

There were no clinical cases of PPR encountered in Kigoma rural and Kasulu districts during the study. On the other hand, 32 clinical cases were observed in Kibondo district. Among these 17 cases were observed during flock visit while 15 cases were reported by respondents in goats to the local veterinary officer. The observed signs included the presence of nodules mostly on the ora-nasal, skin and the genital organs, thick and yellowish nasal discharges (Figure 7 and 8), respiratory distress and lacrimation were observed and shown percentage-wise in Figure 9. Respiratory distress caused by

matting of discharges to the nose were also seen and all affected animals were depressed with hair erected (Figure 10).

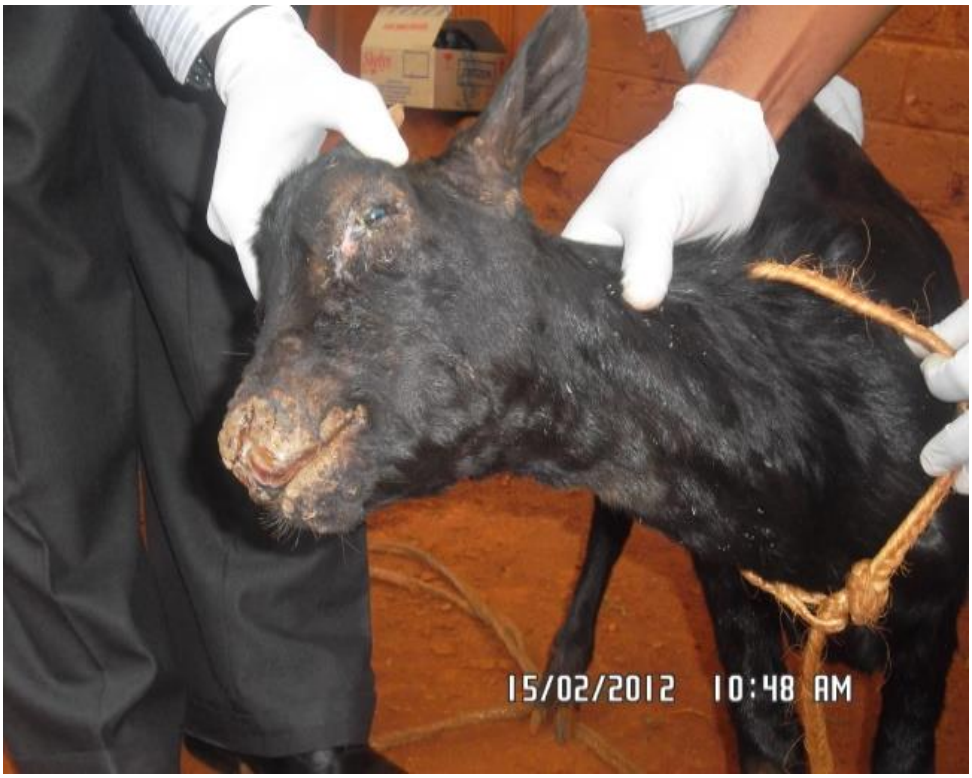

Figure 7: Encrustation and matting on the ora-nasal, ocular region and lacrimation

Figure 8: Encrustation and matting on the muzzle region of the goat

The majority of animals examined had nodules on the skin and genital organs (Figure 9) and less observed signs were respiratory distress in clinically sick animals

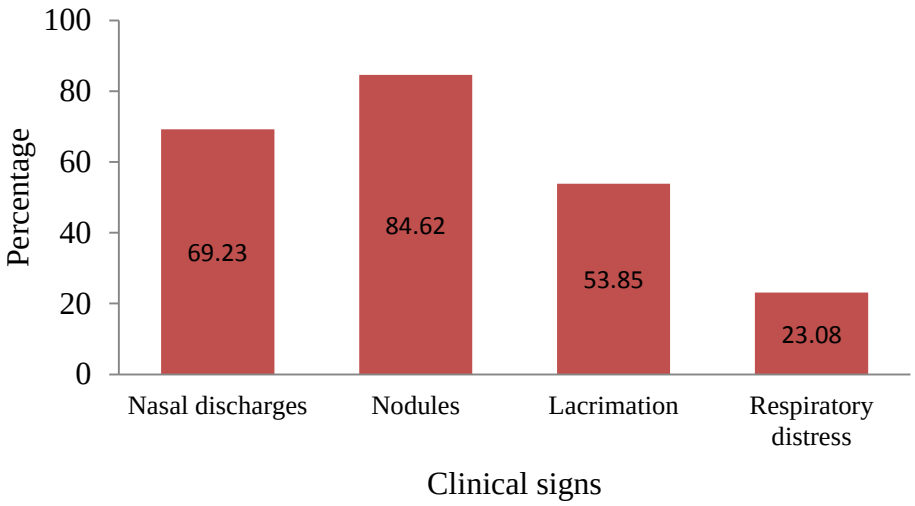

Figure 9: Showing predominant signs shown by sick goats in Kibondo district Kigoma, Tanzania (n=32)

Table 5: Distribution of potential risk factors of PPR startified by study districts from key informants (n=65)

Parameter	%Kigoma rural (n=15)	%Kasulu (n=18)	%Kibondo (n=32)	%Overall (n=65)
Grazing system				
Communal	20.0	61.1	75	55.4
Tethering	80.0	38.9	25	44.6
Watering source				
Surface well	80.0	50.0	71.2	67.7
Water stream	20.0	50.0	28.8	32.3
Source of animals				
Introduced new animals	20.0	16.7	40.6	29.0
Did not introduce animal	80.0	83.3	59.4	71.0
Floor type used				
Raised floor	6.7	5.6	21.9	13.8
Ground earthed	93.3	94.4	78.1	86.2
Age size				
Adults	93.3	94.4	93.7	93.8
Sub-adults	6.7	5.6	6.3	6.2
Contact with cattle				
Yes	66.7	44.4	59.4	56.9
No	33.3	55.6	41.6	43.1

Response from key informants revealed that generally communal grazing was more practised in all districts, although district wise Kigoma rural had lowest percentage (20.0%). The use of shallow surfaced well was common in all districts as a source of drinking water in all districts under study. The majority of respondents preferred houses with ground earth to raised floor because it was easier to make them (86.2%). Other factors are also shown in Table 5.

The economic loss impact of PPR defined by morbidity, crude mortality, case fatality and action taken in Kibondo district, is shown in Table 6.

Table 6: Proportions of the households affected by PPR, the levels of divastation and the action taken by the farmers in Kibondo district Kigoma, Tanzania.

Parameter	No (n)	Total (N)	Percentage
Household affected	22	32	68.8
Morbidity	45	233	19.3
Crude mortality	32	233	13.7
Case fatality	32	45	71.1
Household vaccinated	0	0	0
Sold goats after infection	3	32	9.4
Treated their animal	9	32	28.1

Households affected in Kibondo tethered their affected animals as a way to control animals from spreading the disease to other animals in the communal grazing areas and watering sources (Figure 10).

Figure 10: Tethering of sick goat was practised as a measure to prevent spread of PPR in Kibondo district

CHAPTER FIVE

5.0 DISCUSSION

The present study has confirmed the presence of PPR exposure and infection in western part of Tanzania. It is the first study to describe the existence of seropositive cases in goats and sheep and clinical cases in goats in this region bordering Burundi and DRC. The present study therefore compliments the information on the prevalence of PPR in other parts of Tanzania as reported by Muse *et al*. (2012a) in Southern regions and by Kivaria *et al*. (2009) and Swai *et al*. (2009) in Northern part of Tanzania. Since no animal was vaccinated against PPR, observed seroprevalence in goats and sheep is believed to be attributed to field infection with PPR virus.

The introduction of PPR in western region may be attributed to various practices including introducing new animals in the flock from different locations or other flocks. Similarly, free movements of goats and sheep across the porous international borders between Burundi and Tanzania and between Tanzania and DRC together with the influx of refugees might have also contributed to the introduction of PPR in the study area. In addition, formal and informal small ruminants marketing from neighbouring countries are likely to introduce the disease in the study area. This is supported by results of this study where in Kigoma Rural and Kasulu districts, highest seroprevalence was recorded in border areas with livestock markets. Social transactions like dowry payment, donations, entrustment and barter trade existing in the neighbouring communities along the border with Burundi are associated with movement of small stock across international border, and therefore considered to be among the important factors for the introduction of PPR in the region.

The finding of clinical cases of PPR in Kibondo district only as observed in this study might be explained by the district being the first point of PPR introduction in the region. Efforts made by the district veterinary authority to control the disease might have contributed to protecting other neighbouring districts. It was also noted that the source of clinical cases in Kibondo was from a flock of small ruminants owned by a person who sells small animals. It is speculated that PPR cases started as a single case in Malaragasi street in Kibondo district council after a goat meat seller bought a goat from a goat salesman in the street and brought it home for fattening. The goat later showed clinical signs and after 1 to 2 weeks other goats were affected too in the same flock. The affected goats were sold and others slaughtered for meat illegally. The imposition of quarantine early December 2011 in the affected area by the District Veterinary Officer in Kibondo is believed to be the factor that contributed to the controlled spread of the disease to other villages in Kibondo, Kasulu and Kigoma Rural in the study area.

The seroprevalence of 5.1% of PPR observed in the present study seems to be lower than what was reported in other studies i.e. 31% by Kivaria et *al.* (2009) and Swai e*t al.* (2009) in the northern and 45.8% by Muse *et al*. (2012a) in the southern region of Tanzania. This can be explained by lower population density of small animals stocks and fewer mixed grazing resulting from lower mixing contact among small animals in the study area. Animal management system such as tethering, watering by using bucket and housing used in the study area might have contributed to the lower prevalence. Most small ruminant farmers keep small flocks of goats and sheep which are easily tethered in most of the time and get drinking water by bucket on regular basis. These practices reduce, to some extent, the chances of goats and sheep roaming around during browsing, searching water for drinking and hence the risk of contracting PPR from other flocks is minimised.

The results of the present study further showed higher prevalence of PPR in Kibondo compared to Kigoma rural and Kasulu districts. The higher prevalence of PPR in Kibondo may be influenced by geographical location, relative larger area and a long border along with Burundi and Lake Victoria zone on the northern part. This being the case, there are high chances of many animals being introduced from Burundi to Kibondo before they are moved to Kasulu and Kigoma rural. While the eastern border along DRC is suspected to have seropositive cases although neither clinical case have been reported nor official reports (SADC, 2011). The geographical location of Kibondo exposes this district to free movement of animals. Lack of veterinary services such as vaccination, awareness and knowledge of the disease predispose small ruminants to the disease in this area of study. This fact is in agreement with what was observed by Ahmad *et al*. (2008).

Observation of higher seroprevalence of PPR in goats than in sheep is in agreement with studies by Dhar *et al.*(2002) and Ozkul *et al.*(2002). Similarly, Swai *et al*. (2009) reported the seroprevalence of 49.5% and 39.8 % in goat and sheep respectively in northern part of Tanzania. Abraham *et al*. (2005) also confirmed that goats react more severely to PPR virus exposure compared to sheep and suffer severe clinical cases than sheep. The same trend was observed by Khan *et al.* (2007) in Pakistan, where goats had higher seropositivity (51.2%) than sheep (39.0%). The same findings were reported in north-western Mali by Sangare *et al.* (2007) that seroprevalence in goats and sheep were 44.0% and 34.0% respectively.

The higher prevalence in goats may be attributed to a greater susceptibility of the goat population to infection with PPRv while recovery rate of goats to PPRv infection is considerably less than that of sheep (Dhar *et al*., 2002). However, goats reproduce at

faster rate than sheep, thus replace a large proportion of the goat population per year. Kids become susceptible to PPRv infection at 3–4 months of age (Srinivas and Gopal, 1996), which corresponds with decline natural maternal antibodies (Saliki *et al*., 1993).

Furthermore, the results generally observed more seropositive female (3.7%) than males (1.6%).This finding disagree with that of Rahman *et al*. (2004) and Waret-Szkuta *et al*. (2008) who observed males to be more prone to the disease than females due to genetic factors. However, these findings also contradict those of Swai *et al*. (2009) who observed no significant difference between sex in sheep and goat.

The study has established higher prevalence in adult animals (4.2%) to infection with PPRv than young ones (0.9%). These results are consistent with those by Sow *et al.* (2008), who noted a prevalence of 33.4% in adults compared with 28.0% in young animals. However, Tounkara *et al.* (1996) further noted that the PPR seroprevalence was higher in older small ruminants because in an enzootic area they are more prone to exposure to the virus. This finding is not supported by Singh *et al. (*2004b) who reported different observations.

The present study also observed higher PPR prevalence in small sized flocks (i.e ≤10) (3.1%) than in larger flocks (2.0%). This finding contradict the epidemiological fact that, large sized flock are more likely to be affected than small sized flock due to husbandry practices employed and contacts among animals in the flock and the finding by Somia and Abd, (2012) who observed the same. But more likely the source of animals in these new flocks with fewer animals is from purchase of animals from neighbours or livestock market in which more likely animals with health problems are being sold.

The results further indicated that Kibondo had generally higher seroprevalence (2.0%) and the least was Kigoma rural (1.3%). Although village wise Kalinzi in the far northern western part of Kigoma rural had higher seroprevalence as compared to the rest of the villages in both districts. The higher value may be associated with its geographical location closer to Buhigwe livestock market in Kasulu where illegal movement of animals from Burundi and probably DRC across the lake exist.

In the current study, significant risk factor associated with being cELISA positive was introduction of new animal(s) in the flocks. This is in agreement with the report by (Muse *et al*., 2012a) who reported that introduction of a new animal in the study was associated with the occurrence of PPR in Southern Tanzania.

No significant association between being cELISA positive for PPR and grazing system and animal house ventilation could be established. This could be related to the fact that PPR is transmitted from infected animals to susceptible ones by contact, whether the contact happens at animal housing, watering points, pastures or at both. This finding is in agreement with that of Somia and Abd, (2012) who observed the same association.

In general, PPR being a trans-boundary animal disease of small stocks is masked by perceptions that it has less international trade and public health implications. However, it has direct implications on poverty levels, since small ruminants are mostly kept by rural people with low income generation capacity in most areas. These poor household members have very low capacity to absorb external shocks associated with PPR incursion. School fees payment bills, dowry payment, purchase of different utilities for home consumptions, medical treatment and during harsh condition like prolonged drought and famine are some of items commonly financed by small stock keeping.

Thus, there is a need to ensure good husbandry measures and control activities are in place at various level i.e. village, district, regional and national.

During the disease outbreak in Kibondo district there were some economic losses due emaciation, costs incurred in management and treatment of the affected animals. Reduction in the market value of animals has been also reported as the major loss component as appearance of the animal changes drastically after the illness. Other expenditure cost on medicine, veterinary and labour services has been found to cause more economic loss. Peste des petits ruminants as an emerging disease in Tanzania and neighboring countries and the most important viral infection of small ruminants, is threatening small ruminants' flocks in Africa. Thus, there is a need of implementing strategy for the eradication of PPR in Tanzania and the SADC programme PPR control strategy which are in place. Perhaps, timely joint collaborative efforts for Trans-boundary Animal Disease among the regional countries like vaccination could be the best and low-cost preventive measure to control such deadly disease outbreaks in the region.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Serological findings from this study have established the prevalence of PPR in western areas of Tanzania along DRC and Burundi boarders. Provided that there was no vaccination against PPR done in this area, this finding suggests natural disease cycle in the study area. The source and transmission of the PPR was suggested to be introduction of new animals. The spread of this disease in the western Tanzania pose a high risk of disease spreading to nearby regions of Tabora and Rukwa in the east and southern part respectively and countries of Burundi and DRC on the western borders with unknown PPR status.

6.2 Recommendations

Being an important contagious TADs viral disease, there is a need of implementing already set strategies for combating the disease in the study area in order to reduce economic losses associated with high mortality and condemnation of infected goats, sheep and their products. The community should be educated on basic knowledge of recognizing and reporting clinical cases of sick animals, disease prevention and control methods such as improving animal health and buying animals from recognized livestock markets where animal history can be easily tracked.

Due to the fact that the study was conducted in few villages, the problem might be extreme, thus further studies should be done to ascertain the potential risk factors associated with the introduction and spread of the disease in the region. As a country, epidemiological study should be done in wild ruminants to determine the potential risk

factors associated with the disease spread and call for a national strategy for prevention, control and eventually eradication of the disease in small ruminants and other PPR susceptible and reservoir(s) hosts.

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APPENDICES

Appendix 1: PPR Surveillance Form

FARM LEVEL ENQUIRY

General Information

Region …… District……

Village ……... Sub-village……..

Farm affected…….. GRID Latitude……

REF longitude…….

Information provided by (Name)……. Address……..

Phone no…….……

Name of the owner…….…….

Date of visit…….. Date of outbreak (if any)……

Production details

Type of farm (tick one)

Small scale traditional (<10)

Medium Scale traditional (10-100)

Large scale traditional (100>)

Animal species affected (tick all that apply)

Cattle (…)

Sheep (…)

Goat (…)

Others (specify)

Population information

Species	Present at start of outbreak	No.sick	No. dead	No. destroyed	Slaughtered
Cattle					
Sheep					
Goat					
Other (specify)					

Clinical information

Clinical features	Check for abortion, loss of condition, discharges, diarrhea, high mortality, skin lesion

Vaccinations

Type	Date	Source	Commercial name	Admin route	comments
PPR					
CCPP					
Others (specify)					

Specimen collected …….Village name…….

S/No	Owner	Species	Sex	Age group	Health status

Other observations

……

……

……

Name of investigator……

Signature……..

Appendix 2: PPR RESEARCH EPIDEMIOLOGY FACTORS QUESTIONNAIRE

DATE……

Location: District……..Ward…….Village……

Grid latitude……Ref longitude……..

HH ID……Name of the respondent…….sex

(F/M)…….

Species	Age group	Females	Males	Total
Goat	Pre- weaned			
	Sub-adult			
	Adult			
Sheep	Pre-weaned			
	Sub-adult			
	Adult			

How are your sheep and goat managed?

S/N	Management system	YES	NO
	Tethering		
	Raised Boma		
	Communal grazing		
	Others specify		

How are the housing made?

S/N	Housing system	Yes	No
	Burnt bricks		
	Unburnt soiled bricks		
	Wooden		
	Others (specify)		

How is the floor made up?

	Type of flow	Yes	No
	Raised and wooded		
	Raised but not wooded(specify)		
	Ground floor cemented		
	Ground floor earthed		
	Others (specify)		

Where do animal get drinking water from?

S/N	Water source	Yes/No
1	River	
2	shallow well	
3	Water stream	
4	Any other (specify)	

Awareness of outbreak (Yes/No) seen affected animals (Yes/ No)……

Own animal affected (Yes/No)……. Signs shown by affected animals: (D) Diarrhoea, (L) Lacrimation, N: Nasal discharge, (R) Respiratory distress, (U) Ulcers oral, (n) nodules……

Number of affected animals (current): affected (sick) animals

Species	Age group	Females	Males	Total	Origin of the animal	Cost incurred
Goat	Pre-weaned					
	Sub-adults					
	Adults					
Sheep	Pre-weaned					
	Sub-adults					
	Adults					

Number of animals died in the current outbreak

Species	Age group	Females	Males	Total	Origin of the animal	Cost incurred
Goat	Pre-weaned					
	Sub-adults					
	Adults					
Sheep	Pre-weaned					
	Sub-adults					
	Adults					

When did the current disease start/ observed in your herd?

(Month/Year)……..

Have you seen similar disease in the past (Yes/No)…… If yes,

Species	Age group	Total females	Total males	Affected/ died females	Affected died males	Total affected/ died
Goats	Pre-weaned					
	Sub-adults					
	Adults					
Sheep	Pre-weaned					
	Sub-adults					
	Adults					

When was the disease seen in the past (give month & year): ……

Area where the disease was seen for the first time.

Village:…….....…… ward:……Division:……..

Not sure:……..

Action	Taken: yes/ No	When taken (First time) month/year)
Chemotherapy		
Report to local leader		
Report to livestock Field Officer (LFO)		
Vaccination (Response)		
Others:…….		

Disease still there (Yes/ No)……Knowledge of what disease it was (Yes/ No)……..

Sources of information of what disease it was: 1= extension workers, 2= fellow herders…….

Local name for the disease…....……. Meaning of the local name…….....……

Possible source of infection:

Source	Yes /No	Specify from where (area)
Other animals		
Auction market		
Newly purchased animals		
Not sold animals from livestock market		
Others specify……		

Village has extension workers (Yes/ No)……extension worker inspect animals regularly (Yes/ No)…….

Thank you for your cooperation