SERO-SURVEILLANCE, RISK FACTORS AND MOLECULAR DIAGNOSIS OF *PESTE DES PETITS RUMINANTS* VIRUS IN SOUTH KIVU, DEMOCRATIC REPUBLIC OF CONGO

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ABSTRACT

Peste des petits ruminants (PPR) is an acute viral disease of small ruminants caused by PPR virus (PPRV). For many years, the disease was mainly confined to West and Central Africa but has now spread southwards to previously PPR-free countries. The disease was first reported in Democratic Republic of Congo (DRC) in 2012. The disease causes high morbidities of up to 100 % and mortality rates between 50 and 90% in domestic small ruminants (goats and sheep) leading to the socio economic loss impact to the farmers. This study aimed to perform molecular diagnosis and sero-prevalence of PPR associated with transmission risk factors in unvaccinated sheep and goats from South-Kivu in province in DRC using respectively reverse-transcriptase polymerase chain reaction (RT-PCR), competitive enzyme-linked immunosorbent assay (cELISA) and a structured questionnaire. The results showed with cELISA an overall seroprevalence of 28.5% (n=319), out of which 11.3% and 32.7% seropositivity was found in sheep and goats, respectively. *Peste des petits ruminants* seroprevalence was higher in the territories that recorded high rainfall, 34.5% (n=142) in Shabunda and 29.4% (n=79) in Mwenga. In a total of 11 risk factors investigated four were found to be associated with PPR seroprevalence ($p \leq 0.05$). Among them we have animal's age (OR: 9.34), grazing and farming system (6.28), territory geographic location (OR: 5.1) and the animal's origin (OR: 0.5). *Peste de petits ruminants* seroprevalence was higher in small ruminants kept in communal grazing system (30.6%) and free ranging system (31.2%). Sheep and goats of >12 months had a significantly higher PPR seroprevalence (35.1%). As no PPRV RNA was detected in any of the blood collected using reverse transcriptase polymerase chain reaction (RT-PCR), we recommend further studies to be focused on molecular characterization and isolation of PPR virus.

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DECLARATION

I, **Bwihangane Birindwa Ahadi**, 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 that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

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The declaration is hereby confirmed;

_____ _____

Prof. Gerald Misinzo Date

(Supervisor)

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Finally, I wish to thank my family for their moral support, prayers and encouragement during my studies. To Charline, my wife, Nathan my son and all my coming children, may you find my gratefulness through this word.

DEDICATION

To my wife Charline Bwenge Rwananza, my parents, Mr. Ernest Birindwa Musafiri and Ms. Emmerciane Nabintu Busomoke, my brothers and sisters for their love, encouragement and support, and to the children that God will give to me.

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LIST OF ABREVIATIONS AND ACRONYMS

µl	microliter
BLAST	Basic Local Alignment Search Tool
Bp	base pair
cELISA	competitive enzyme-linked immunosorbent assay
DIVA	differentiation of infected and vaccinated animals
DNA	deoxyribonucleic acid
F	fusion protein
FAO	Food and Agriculture Organization
H	haemagglutinin protein
M	matrix protein
Mg	milligram
Min	minutes
Ml	milliliter
mRNA	messenger ribonucleic acid
N	nucleoprotein
NCBI	National Center for Biotechnology Information
NP3/NP4	nucleoprotein primer set
^{o}C	degree celsius
OIE	Office International des Epizooties
P	phosphoprotein
PCR	polymerase chain reaction
PPR	*peste des petits ruminants*

PPRV	*peste des petits ruminants* virus
RdRp	RNA-dependent RNA polymerase
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
SADC	Southern African Development Community
TAD	transboundary animal disease
TAE	Tris Acetic EDTA buffer
WHO	World Health Organization of the United Nations

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Peste des petits ruminants (PPR) is one of the acute contagious and viral epizootic diseases of domestic (sheep, goats) and wild (springbuck, gazelles and impala) small ruminants caused by PPR virus (PPRV) which belongs to the family *Paramyxoviridae*, Subfamily *Paramyxovirinae*, order *Mononegavirales* and genus *Morbillivirus*. This virus belongs to the same familyas the rinderpest virus (RPV), canine distemper virus (CDV) and measles viruses (MV) (Luka *et al.*, 2012). It has been reported by Banyard *etal*. (2010) that PPR is highly infectious and causes high mortality. Abubakar *et al.*(2011) found that cattle are able to seroconvert in case of a high prevalence of PPR in small ruminants, although they are not susceptible to the disease. PPR is a transboundary animal disease (TAD) and is attracting attention especially in the Southern African Development Community (SADC) due to its occurrence in explosive form, high mortality and rapid spread across national borders or even across continents (Kayunze *et al.,* 2012). The disease is seen as a major threat to domestic small ruminants production and thereby influences the livelihoods of poor farmers. This is because sheep and goats, also known as “cattle of the poor”, are among the main farm animals owned by the poor in most developing countries. They serve as sources of not only milk and meat for family consumption, but also for income that can easily be mobilized for paying household expenditures(FAO-EMPRES, 2009).

Serological data from Nigeria revealed that antibodies occur in all age groups from 4-24 months indicating a constant circulation of the virus (Taylor, 1979). The transmission of PPR is achieved by direct contact from infected to susceptible animals by close contact or through respiratory and oral routes (Muse *et al*., 2012a). Clinically, the disease is characterized by proliferative and self-resolving lesions around the muzzleand lips of involved animals, serous nasal and ocular discharge which become mucopurulent (Zhao *et al.*, 2010).

In Africa, *peste des petits ruminants* was first recognized as a contagious "rinderpest-like" condition in goats and was originated in Western Africa, spread to Asia and then to Sudan and Ethiopia (Swai*et al.,* 2009). It is believed that PPR might have been introduced in African countries through the movement of live infected animals (Kaukarbayevich, 2009). The South-Kivu region of the Democratic Republic of the Congo (DRC) is believed to have been infected since 2008, when outbreaks based on clinical signs were reported, but no any laboratory diagnosis (molecular or serological) has been done to confirm the cases. An estimated one million goats and 600 000 sheep are at risk of contracting PPR, representing one-quarter of goats and two-thirds of sheep throughout the country (FAO and OIE, June 2012). Previous studies show that goats are severely affected than sheep which generally undergo a mild form(Kayunze *et al.,* 2012;Ismail and House, 1990)

1.2 Problem statement andstudy justification

It is impossible to ascertain the magnitude and variability of a disease within susceptible populations without efficient diagnosis, which may lead to failure of the

eradication program (Banyard *et al.*, 2010). *Peste des petits ruminants* is a highly contagious and economically important disease of small ruminants (Chauhan *et al.,* 2009). The Southern African Development Community (SADC) report in 2012 shows that, the DRC reported that since its emergence in 2010 to June 2012, PPR had caused the death of almost 120000 small ruminants. It is estimated that the direct loss, i.e. value of dead sheep and goats, to be US$5.3 million (SADC, 2012). This estimate does not take into account of the socio-economic impact and other benefits of goats andsheep to the smallholder farmers. Trans-border movements of cattle, goats and sheep for breeding in neighboring countries, farming systems, trade and other socio-economic functions could be the main predictor for introduction, transmission and maintenance of PPRV in some areas that are still considered to be endemic. According to TADs Steering Committee for Africa (2012) during the years 2008-11 a total of 4 079 outbreaks, 431 258 cases and 56 663 deaths due to PPR were reported in 25-30 (56%) countries in Africa.The disease not only threatens food security in these countries, but could also result in a spill-over to southern African countries that have never had the disease. Morbidities of up to 100% and mortality rates between 50 and 90% are common (Rossiter, 2004). In severe outbreaks affected goats may die within ten days of exposure to the virus.Eliminating PPR is seen as key to poverty reduction in Africa, because sheep and goats are generally kept by the poorest farmers, who have the least ability to absorb the loss of one of their few assets. Goats and sheep are species of choice of pastoralists in DRC (around 80% of farmers), they are recognized as ready sources of food and cash across sub-Saharan Africa and are also an important animal protein source (meat and milk).

Any seroprevalence and molecular diagnosis of PPRhave been conducted in Eastern part of DRC while this region is considered to be at high risk by SADC(Muse *et al.,* 2012a). Anticipatively the findings of this study will provide information on risks factors associated with PPR transmission, PPR serological status and undertake molecular diagnosis of PPRV in South Kivu province for rational control measures against the disease.

1.3 Objectives

1.3.1 Overall objective

The main objective of this study is to determine the PPRVantibody prevalence, diagnose the virusin small ruminants (goats and sheep) population for epidemiologic control and identify risk factors associated with PPR transmission in reported outbreak cases in South Kivu Region of DRC.

1.3.2 Specific objectives

i. To evaluate the prevalence of PPRV antibodies in reportedcases of South Kivu region in order to identify the risks areas for epidemiological control,
ii. To perform molecular diagnosis of PPR in goat and sheep based on Nucleoprotein (N) and Fusion protein (F) genes, during a suspected outbreak in South-Kivu, and
iii. To identify the risk factors related to PPR transmission in selected areas of South-Kivuregion where the outbreak has been reported in DRC.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Etiology of *peste des petits ruminants*

Several early studies showed the close relationship betweenPPR and rinderpest virus viruses (RPV)and for a time PPRV was considered to be a host-adapted variant of RPV (Mornet *et al.,* 1956; Gilbert and Monnier, 1962; Whitney*et al.,* 1967). The observations in the fieldshowed that the disease is independent of RP andotherMorbilliviruses (includingmeaslesand canine distemper).Studies with monoclonal antibodies and nucleic acid techniques confirmedthe distinction between the two viruses, and that PPRV was acompletely separate pathogen (Diallo*et al.,* 1989, 1990).Rinderpest virus is the direct descendent of an ancestral Morbillivirus from which the others evolved. It diverged from RPVmore recently than did measles and canine distemper viruses (McCullough*et al.,*1986). If PPR originated in West Africa in 1942, the virus must have evolved either after the 1887-96 RP pandemic or fromstrains of RP already established in Africa long before the infamous pandemic. Since neither optionseems likely, an alternativemight be that PPR originated in Asia and was independently introduced into Africamuch asRP was introduced through infected livestock. Nucleic acidsequencing data, however, clearly distinguishes Africanrinderpest virus isolates from those outside Africa,and it has beensuggested that PPRmay have evolved fromthe adaptation of RPV to small ruminants on at least two separate occasions, in Africa and Asia respectively(Taylor*et al.,* 1990).

2.2 An overview of *peste des petits ruminants*

The *Peste des petits ruminants*, an acute contagious viral infection of both wild (springbuck, gazelles and impala) and domestic(sheep and goat) cloven-hoofed small ruminants, is characterized by fever, pneumonia, profuse diarrhea, and inflammation of the mucous membrane of the respiratory and digestive tracts (Ismail and House, 1990). Although all wild ruminants are susceptible to infection, PPR has only been diagnosed inGazellinae (Dorcas gazelle), Caprinae (Nubian ibex and Laristan sheep), Hippotraginae (Gemsbok) and Capra aegagrus blythi (Sindh Ibex) (Abubakar *et al.*, 2011).*Peste des petits ruminants*was first detected in 1942 when Gargadennec and Lalanne realized that rinderpest which is a like disease caused symptoms in sheep and goats but was not transmittable to cattle (Gargadennec and Lalanne, 1942). Currently, comparison of various characteristics between PPR and RP, rescuing the chimeric viruses for differentiation of infected and vaccinated animals (DIVA) and full genome sequencing of PPRV, have made substantial advances in understanding of the disease.

2.3 *Pestedes petits ruminants* virus

2.3.1 Taxonomy

Peste des petits ruminants virus belongs to the family *Paramyxoviridae*, order *Mononegavirales* and is a member of the genus *Morbillivirus* along with the rinderpest, canine distemper and measles viruses, porpoise and dolphin distemper viruses that infect marine mammals(Chauhan *et al.,* 2009).

2.3.2 Genome structure

*Peste des petits ruminants*virus is pleomorphic in shape and isenveloped and enclosed in a ribonucleoprotein core together with nucleocapsid protein (Fig. 1). The genome is composed of 15 948 nucleotides, which is the longest of all the *Morbillivirus* members (Bailey *et al.*, 2005).

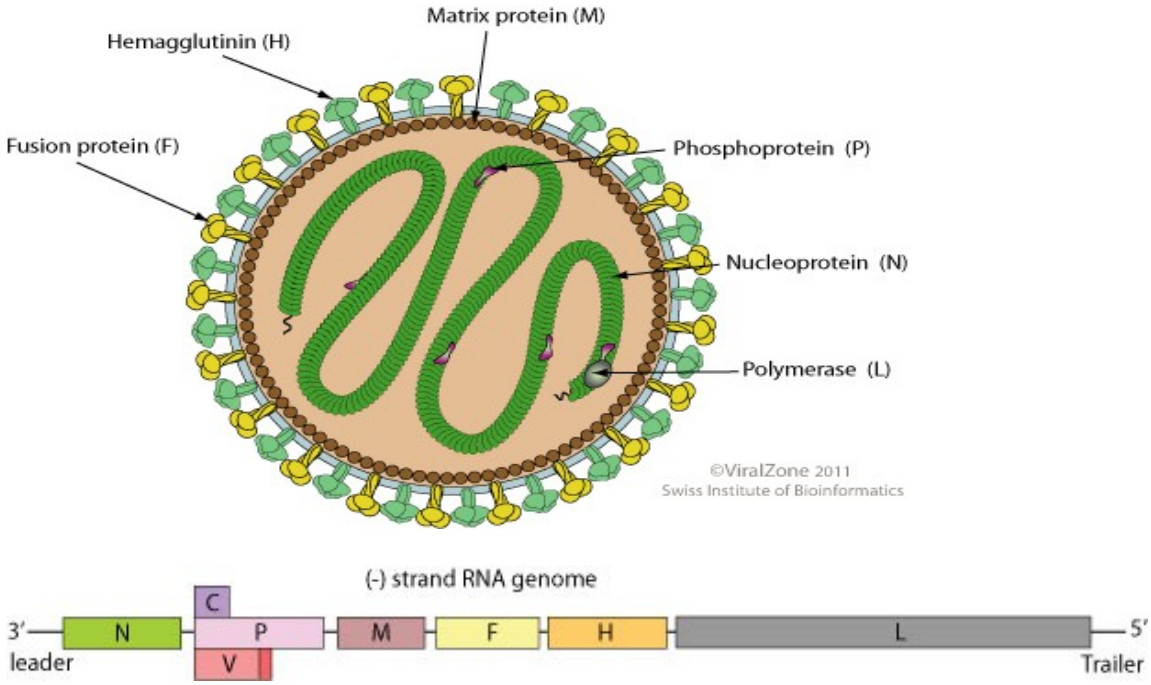

Figure 1: *Peste des petits ruminants* virus virion and genome orientation.The PPRV genome encodes sixgenes, each responsible for transcription of a single protein in the order nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin (H) and the large RNA polymerase (L). The P gene encodes for two additional non-structural proteins, C and V(Bailey *et al.*, 2005).

The three viral proteins (M, F and H) are associated with the host-derived envelope (Luka *et al.,* 2011).The M protein is linked to the nucleocapsid and surface proteins (F and H) and the L protein acts as RNA-dependent RNA polymerase. The association of P protein to N and L is linked to viral cycle control, transcription and translation regulation.

The genes are in the order of 3' N–P/C/V–M–F–H–L 5'.The non coding regions include a 3' leader 50 nucleotides in length which acts as a transcriptional promoter, a 5' trailer sequence 50-161 nucleotides long and inter-genomic regions between each gene which are three nucleotides long in all members of the genus *Morbillivirus* including PPRV, the N protein is the most abundant viral protein due to its presence at the extreme 3′-end of the viral genome (Viralzone, 2011).

2.4 Host response to PPRV

The protective immune response is usually elicited against the surface F and H proteins of PPRV. However, among the viral proteins most of the neutralizing antibodies are directed against the H protein during PPRV infection (Diallo *et al.*, 2007). Owing to its high quantity during infection, the N protein is considered the most immunogenic, but the immunity produced against N protein does not protect the animals from the disease. The H and N proteins remain the most acceptable targets for the design of PPRV diagnostic tools (Munir*et al.,* 2011).

2.5 Potential for PPR serodiagnosis

Based on putative amino acid sequences, the N protein is divided into four regions (I, II, III, and IV). Region I includes amino acids 1-120, II from 121 to 145, III comprises 146-398, and IV finishes with 421-525. The first two regions are reported to be more immunogenic than the other two. It has further been summarized that there is development of earlier immune response to region I and II than region III and IV (Bodjo *et al.*, 2007). Most of the diagnostic assays for PPRV have been developed based on monoclonal antibodies (mAb) raised against the N protein. The H protein of PPRV, on the other hand, is the most diverse among all the members of *Morbilliviruses*. Since the H protein determines the cell tropism, most of the protective host immune response is raised against the H protein (Renukaradhya *et al.*, 2002). Mapping of the functional domain, using mAb, has demonstrated that two regions, one at amino acids 263-368 and other at 539-609, are the most immuno-dominant epitopes (Seth and Shaila, 2001).

2.6 Transmission and pathogenesis

Transmission of PPR is achieved by direct contact from infected to susceptible animals by close contact or through respiratory and oral routes.Some transmissions cases by aerosol or via handling contaminated animal products were described by Nussieba *et al.*(2009).The pathogenesis of PPR is assumed to be essentially similar to that of RP. Infection probably occurs via the oropharynx with subsequent virus multiplication in the draining lymph nodes. The ensuing viraemia transports the virus to other lymphoid tissues and susceptible epithelia throughout the body, where it multiplies, cause the cytopathology responsible for the development of lesions and

disease. Virus and viral antigens can be detected in blood, body secretions and lymphoid organs during the early stages of clinical disease. The titers of infectivity can reach 4 $\log_{10}$ $TCID_{50}$ in oral, nasal, ocular and pharyngeal swabs and 5 $\log_{10}$ $TCID_{50}$ per gram of faeces (Abegunde and Adu, 1977). During the later stages of disease both infectious virus and antigen are increasingly difficult to detect, probably as a result of the rising antibody levels. Counter-immuno-electrophoresis (CIEP) can detect precipitating antibody as early as three days after infection in some animals, although most have detectable antibodies only after seven or eight days (Durojaiye and Taylor, 1984).

2.7 Life cycle of PPRV

Peste des petits ruminants virus life cycle starts with the attachment of haemagglutinin to the cell-surface receptors, then fusion of the virion envelope with cellular membranes occur and then the nucleocapsid virus is released into the cytosol of infected cell (Chauhan *et al.,* 2009).The virus polymerase binds to the single promoter located at the 3' end of the genome (Barrett *et al.,* 2006). It partially uncoats the nucleocapsid and transcribes the genesinto positive stranded mRNAs which are then translated into structural and non-structural proteins (Lefèvre and Diallo, 1990). Transcription either terminates after a gene or continues to the next gene downstream, which means that genes close to the 3' end of the genome are transcribed in the greatest abundance, whereas those toward the 5' end are least likely to be transcribed, a phenomenon known as transcriptional gradient (Cann, 2005). Because the N gene is located at the 3'end of the genome, it is the most expressed gene (Kwiatek *et al.,* 2010). The N concentration in the cell then

determines when the L switches from gene transcription to genome replication (Barrett et *al.,* 2006). Replication results in full length, positive stranded antigenomes that are in turn transcribed into negative-stranded virus progeny genome copies (Cann, 2005). Newly synthesized structural proteins and genomes self-assemble and accumulate on the cell membrane, bud off from the cell and in the process gaining their envelopes from the cellular membrane they bud from.

2.8 Diagnosis of PPRV

2.8.1 Clinical diagnosis

Although a tentative diagnosis of PPR can be proffered based on clinical signs, laboratory confirmation is required for differential diagnosis from other diseases with similar signs. Clinically, the disease is characterized by proliferative and self-resolving lesions around the muzzle (which becomes dry) and lips of involved animals, serous nasal and ocular discharge which become mucopurulent (Zhao *et al.*, 2010). Ismail and House, (1990) found that PPR is characterized by fever, pneumonia, profuse diarrhea and lameness, inflammation of the mucous membrane of the respiratory and digestive tracts. Scabsor nodules can be seen on the lips, the small intestine is congested and hemorrhages may be present, lymph nodes associated with lungs and intestinal tract are soft, swollen and focally or diffusely congested (Rossiter, 2004). The symptoms of PPR are very similar to those of rinderpest, only the formation of small nodular skin lesions on the outside of the lips around the muzzle and the development of pneumonia during the later stages of the disease are seen in PPR but not in rinderpest. On Post mortem, the carcass is usually emaciated and soiled with soft/watery feces.

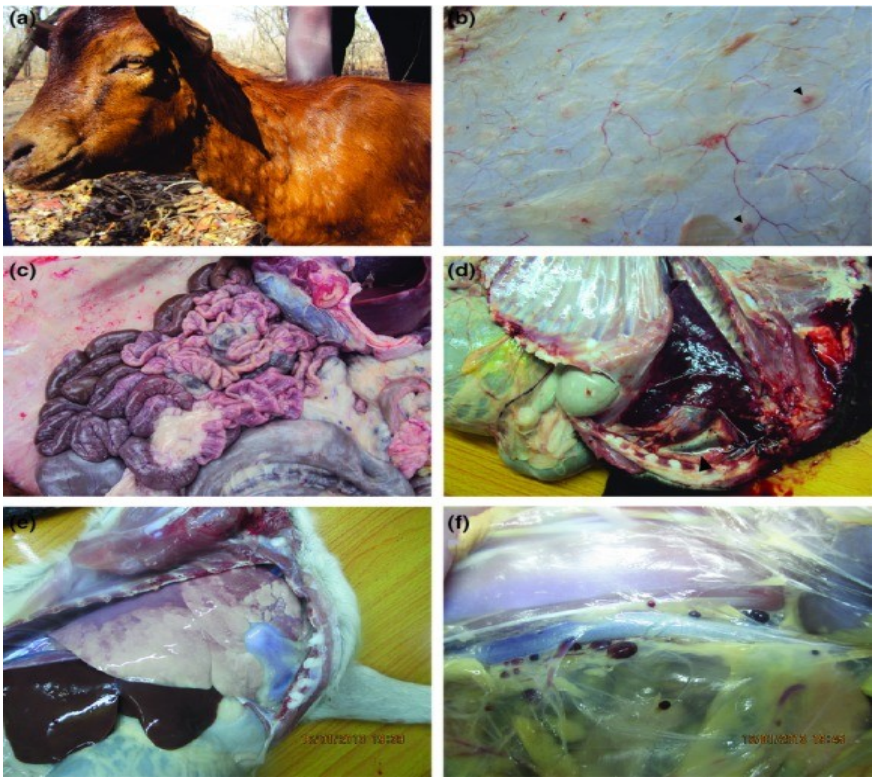

Figure 2: Clinical signs and post mortem features in goats with peste des petits ruminants.(a) A goat with oculonasal discharges, periorbital edema and cutaneous nodules. (b) After skinning, cutaneous nodules were mainly confined within the skin with the exception of a few cutaneous nodules that could be observed below the skin (arrow head). Other postmortem findings in goats with PPR included congestion of intestines (c), pneumonia (d and e) and froth formation (d, arrow head) and hemorrhage of the lymph nodes draining internal organs within thoracic and abdominal cavities (f) (Kgotlele *et al.,* 2014).

Disease severity and the clinical signs depend on various factors: PPRV lineage, species, breed and immune status of animals. So a definitive diagnosis of PPRV infection cannot be based on clinical impressions alone, but must rely on laboratory confirmation.

2.8.2 Laboratory diagnosis

Successful implementation of control measures for PPR requires rapid, specific and sensitive methods for diagnosis. Small ruminants infected with PPR are routinely diagnosed on the basis of clinical examination, gross pathology, histological findings and laboratory confirmation (Atta-ur-Rahman *et al.*, 2004; Bruning-Richardson *et al.*, 2011). A number of serological and molecular diagnostic tests are used for the detection of PPR virus.Conventional techniques used for PPRV detection are: agar gel immunodiffusion (AGID) (Munir *et al.*, 2009b, CIEP (Diallo *et al.*, 1995), dot enzyme immunoassay (Perl *et al.*, 1995), differential immuno-histochemical staining of tissue sections (Saliki *et al.*, 1994), haemagglutination (HA) and haemagglutination inhibition (HI) tests (Saravanan *et al.*, 2006; Raj *et al.*, 2008), virus isolation (Brindha *et al.*, 2001; Manoharan *et al.,* 2005), competitive enzyme-linked immunosorbent assay (c-ELISA) (Anderson *et al.*, 1991; Ezeibe *et al.*, 2008), novel sandwich ELISA (Anderson and McKay, 1994; Munir *et al.*, 2009b), immuno-capture enzyme-linked immunosorbent assay (IC-ELISA) (Libeau *et al.*, 1994; Singh *et al.*, 2004; Khan *et al.*, 2007; Abubakar *et al.*, 2008; Saravanan *et al.*, 2008), immunofiltration (Diop *et al.*, 2005), and latex agglutination tests (Keerti *et al.*, 2009).Conventional techniques such as AGID cannot be used for routine diagnosis, as these are less sensitive and not reliable (Osman *et al.*, 2008). However, HA and HI tests, being simple, cheaper and comparatively sensitive, can be used for routine screening purposes in control programmes (Osman *et al.*, 2008; Munir *et al.*, 2009). For quick diagnosis and control measures, pen-side tests such as chromatographic strip test (Hussain *et al.*, 2003; Aslam *etal.*, 2009) and dot ELISA that can be performed without the need forequipment or technical expertise, are

highly desirable (Hussain *et al.*, 2003). Virus isolation in cell culture can be attempted using several different cell lines. Although Vero (African green monkey) cells have been the choice for isolation and propagation of PPRV, it is reported that B95a, an adherent cell line derived from Epstein-Barr virus-transformed marmoset B-lymphoblastoid cells, is more sensitive and supports better growth of PPRV lineage IV as compared to Vero cells (Bruning-Richardson *et al.*, 2011). Techniques for virus isolation cannot be used as routine diagnostic tests as they are time-consuming and cumbersome (OIE, 2008).

Serological tests include virus neutralization and competitive ELISA assays. Both tests can distinguish PPR from rinderpest; this is not always possible with older serological tests such as complement fixation. Enzyme linked immunosorbant assay using monoclonal antibodies have been used for serological diagnosis and antigen detection for diagnostic and screening purposes. For PPR antibody detection, competitive ELISA is a better choice as it is sensitive, specific, reliable, and has a high diagnostic specificity (99.8%) and sensitivity (90.5%) (Choi *et al.*, 2005b; Sreenivasa *et al.*, 2006). Immunocapture ELISA is a rapid, sensitive and virus specific test for PPRV antigen detection and it can differentiate between RP and PPR viruses. Moreover, it is more sensitive than AGID (Abraham and Berhan, 2001).Detection of PPRV specific antibodies in serum is the standard test for the rapid laboratory diagnosis of PPRV. Antibody testing is most commonly performed using commercial enzyme linked immunoassay (ELISA) kits. For PPR antibody detection, cELISA is a better choice as it is sensitive, specific, reliable, and has a high diagnostic specificity (99.8%) and sensitivity (90.5%). It can also distinguish Peste des petits ruminants from Rinderpest (Sreenivasa *et al.*, 2006).Systems for the

direct detection of PPRV through RT-PCR are becoming more common and, although standard methods are becoming established, no single standard method has yet been developed.

2.9 Geographical distribution of PPR

2.9.1 World distribution

*Peste des petits ruminants*were first described inWest Africa in the 1940s and particularly in Côte d'Ivoire (Gargadennec and Lalanne, 1942) and thereafter, it has been recognized in many of the sub-saharian countries that lie between the Atlantic Ocean and the Red Sea (Lefèvre and Diallo, 1990).*Peste des petits ruminants* virus is epidemic in sub-Saharan Africa, the Middle East and Asia (Dhar *et al.,* 2002; Ozkul *et al.,* 2002; Kwiatek *et al.*, 2007; Kerur *et al.,* 2008). Viruses from 27outbreaks in Asian and Middle Eastern countries, reported between 1993 and 2000, and two recent outbreaks from the African continent were compared with the prototype African strand.The proper understanding of lineage distribution in a specified region is essential when choosing the appropriate homologous prototype to ensure efficient immunization. The continued application of heterologous vaccine candidates hitherto not prevalent may lead to generation of novel lineages, or allow the existing population to evade protection, especially in RNA viruses.

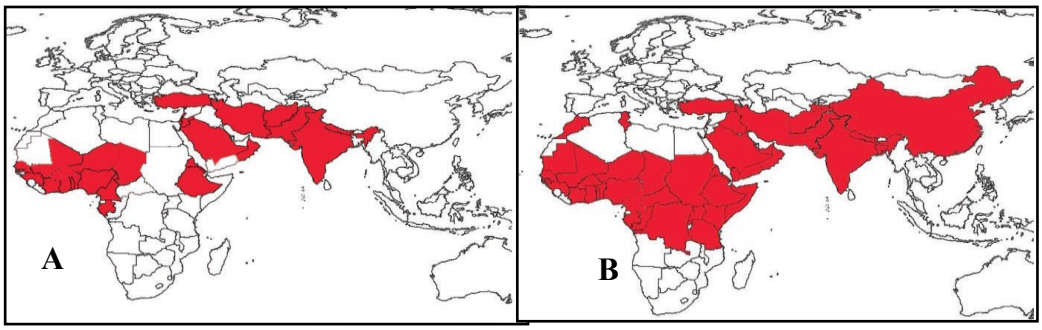

Figure 3: World distribution and spread of PPR from (A) 1998 to 2000 and (B) 2008 to 2010.The red colour shows countries where PPR cases have been reported. The disease was absent in DRC from 1998 to 2000 (Fig. 3 A), but then from 2008 to 2010 (Fig. 3 B) outbreaks have been reported. Source: Baron *et al*., 2011.

The identification of the lineage is a pre-requisite for fruitful diagnosis, epidemiology and control.Phylogenetically, based on the fusion (F) and nucleocapsid (N) genes, PPRV can be classified into four distinct lineages (Shaila *et al.*, 1996). PPRV belonging to lineages I and II are exclusively isolated from the countries of PPRV origin in West Africa. Lineage III is restricted to Middle East and East Africa, although some of the viruses that belong to lineage III have also been isolated from southern India. Lineage IV is considered to be a new lineage comprising newly emerging viruses, and is most prevalent in Asian countries (Shaila *et al.*, 1996; Munir *et al.*, 2011).

Of the four known lineages of PPR virus, lineage I and II viruses have been found exclusively in West Africa, but virus from an outbreak in Burkina Faso in 1999 fell into the lineage 1 group. Viruses of lineage III have been found in East Africa, where an outbreak in Ethiopia in 1996 was of this type.

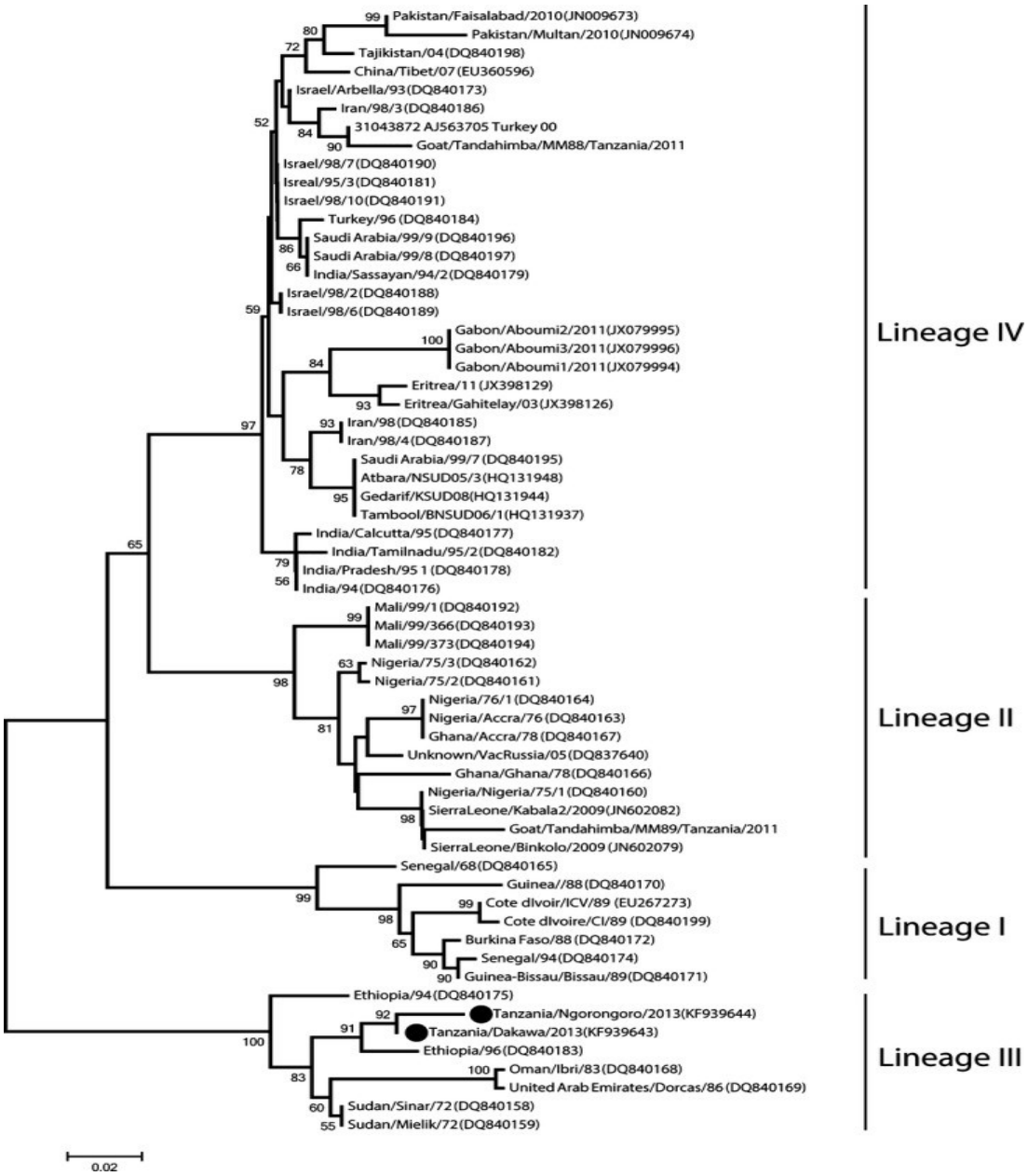

Figure 4: Phylogenetic relationship of *peste des petits ruminants* viruses showing theknown distribution of four different lineages**.**

Source:Kgotlele*et al.,* 2014.

Pronab reported also in 2002 that in the past eight years virus of exclusively the fourth lineage has spread across the Middle East and the Asian sub-continent.

2.9.2 PPR status in DRC and neighboring counties

The Democratic Republic of Congo reported that since its emergence in 2010 to June 2012, PPR had caused the death of almost 120 000 small ruminants (SADC,2012).*Peste des petits ruminants* is a transboundary animal disease; therefore, there is an obligation to inform neighbouring countries and the international community of its absence or presence in a country (Wambura, 2000).

This delay in the confirmation of PPR underlines the importance of having efficient disease surveillance and diagnostic capacity especially for emerging diseases of animals (Muse *et al.,* 2012a).

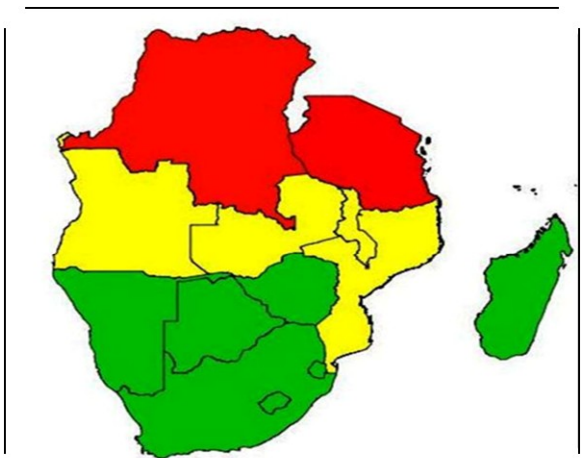

Figure 5: Southern African Development Community distribution of PPR.

The SADC report in 2012 ranked DRCamong infected countries together with Tanzania (shown with red color in Fig.5) while countries sharing a boarder with the above infected (Angola, Zambia, Malawi and Mozambique) are classified as high risk areas (shown in yellow in the Fig.5). The rest of SADC zone (in green color onFig.5) are known as low risk areas(SADC, 2012)

2.10 Controlof *peste des petits ruminants*

Sick and infected goats and sheep should be isolated fromother stock and effective quarantine imposed until at least onemonth after the complete recovery of the last clinical case.Strict control of animal movement is however often difficult to achieve.

Vaccination is therefore themosteffective form of control for PPR. Since the early observations of cross-immunity betweenPPR and RP several workers have used attenuated RPV vaccine to protect sheep and goats against PPR (Bonniwell, 1980; Marinere*t al.,* 1993).This vaccine is safe in pregnant goats and confers clinicalimmunity against challenge with virulent PPRV for at leastthree years, although its longer-term duration is uncertain.In the past, when a homologous vaccine against PPR was not available, a heterologous live attenuated tissue culture rinderpest vaccine (TCRP) (based on the antigenic similarity of PPRV with RPV) was used to control PPR.

Kids born to damsthat have been immunized with RPV have heterologous colostral antibodies to PPRV for up to threemonths,predictably shorter than the four to eightmonths usually describedin calves with homologous maternal immunity to RPV (Brown, 1958).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

Democratic Republic of Congo is located in central side of the African continent.South Kivu province where this study was conducted is located in Eastern part of DRC and borders the provinces of North Kivu to the North, Kivu lake North East, Maniema to the West, and Katanga to the South. Itshares its borders with the countries of Burundi, Rwanda and Tanzania in the East. The area is about 65 070 km^2, total population size of 4 614 768 (71persons per km^2). Koppen-Geiger Climate classification systems classify its climate as tropical wet and dry (Aw) and the altitude is 1531 m above the sea level with an average rainfall of about 1 500 mm with more than 50 % of the total land used for grazing. In South Kivu, goats and sheep are estimated to be respectively one million and 600 000 (FAO, 2012).

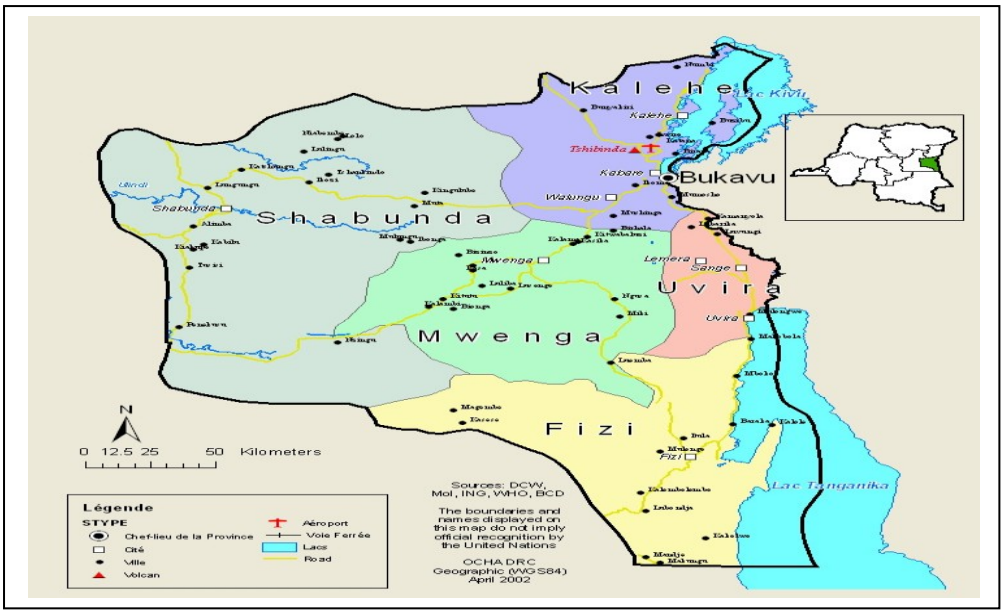

Figure 6: Map of south Kivu Region showing thesampling areas.

This province is composed of seven territories that are Fizi, Kabare, Kalehe, Mwenga, Shabunda, Uvira and Walungu. This study was carried out in 16 selected villages within threeterritories known aspastoralist area of South-Kivu in DRC where PPR outbreaks have been reported. These territories are located in the different geographicpositions: Shabunda (<1 000m), Mwenga (1 500-3 000 m), and Fizi (1 000-1 500 m of altitudes).

3.2 Farms of goat and sheep in study area

Different farms were visited in order to identify the farming and grazing systems used in south-Kivu province.

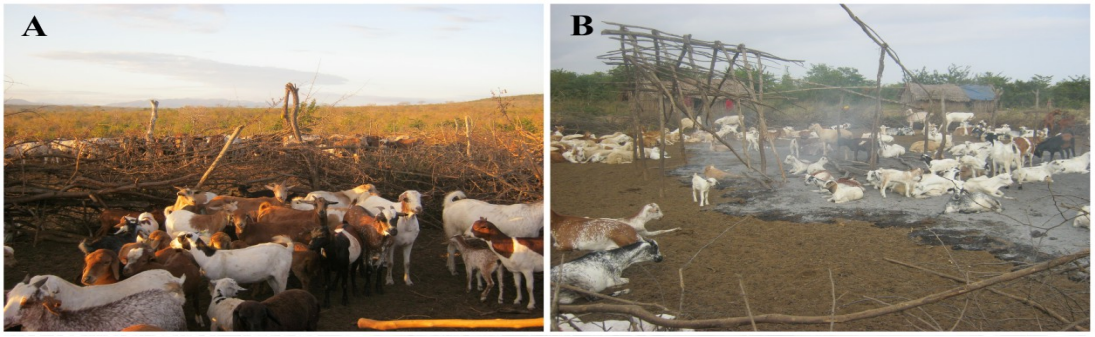

Figure 7: Farms of goats and sheep in communal grazing system in the study areas of South-Kivu.The two pictures A and B show two farms where the animals (goats and sheep) are feeding in communal feeding system. This may lead to the high risk of disease transmission from infected animal to another.In total 65 animals were selected for sampling purposively based on clinical signs for molecular diagnosis and 319 samples were randomly selected in different farms selected purposively for detection of antibodies against PPRV in study areas.

3.3 Study design and data collection

This study employed a cross-sectional study design with purposive sampling of animals within the PPR reported areas in South-Kivu Region. Samples for serology analysis were collected randomly from villages in targeted regions and then samples for molecular analysis were collected in villages with suspected PPR cases based on reports from local veterinary officials and farmers. Samples were collected from September 2014 to January 2015. Randomly selected goats or sheep were subjected to clinical examination prior to sample collection. Animals which showed clinical signs suggestive of PPR were purchased and sacrificed for post-mortem examination and sample collection. The age groups of sampled animals were in the range of 1-

4months to rule out maternal antibody (from 5-12months) and to discover recent infection (>12months) and only unvaccinated animals (sheep and/or goat) were considered for serological analysis.

3.4 Sample size determination

Territories and Districts were selected purposely in clusters based on outbreak report and then a random selection of animals within the selected villages was conducted. For sera-collection, an estimated sample size based on priori prevalence from the Rapid Epidemiological Assessment study described earlier p =22.1% (Kirkwood B. and Sterne J., 2013) according to the formula: $n = z_{\alpha}^{2} \times [p \times (1\text{-}p)/L^{2}]$ was used,

Where; $z_{\alpha} = 1.96$, $\alpha = 0.05$, $p = 0.221$, $L = 0.05$; L is the desired level of precision or accuracy, n is the sample size, D the design effect and N the corrected Sample size. An average number of animals per village, m=6 with a Design effect, D=0.936 was computed according to: $D = 1 + (m - 1) \times p$. The sample size was now corrected to be 319 serum samples for serological analysis by the design effect value using the formula, n_{new} =N= $n \times$ D with a precision of 95% confidence. A large number of goats were considered due to their high susceptibility to the disease compared to sheep. For molecular analysis, the sample size was opportunistic according to the PPR suspected animal based on the clinical signs.

3.5 Sample collection and preparation

3.5.1 Samples for serological analysis

Blood was collected by jugular vein puncture using sterile vacutainer tubes of 5 ml(BD Biosciences, Franklin Lakes, USA) and left to clot overnight at room

temperature and left to clot overnight at room temperature for serological test. Serum was decanted into sterile cryovials and kept on ice during transportation to the laboratory. In the laboratory, sera were stored at -80°C. Each tube was labelled using codes describing the village and/or district and the number of the questionnaire where several parameters for risks factors analysis were collected.

3.5.2 Samples for molecular analysis

A total of 65 bloods samples from the jugular vein were collected from small ruminants showing acute clinical signs for molecular diagnosis of PPRV using RT-PCRwith primers derived from the N and F genes (Fig.6a and b). Approximately 4 ml of whole blood sample was collected from the jugular vein of each animal using vacutainer tubes containing anticoagulant, EDTA (BD Biosciences, Franklin Lakes, USA) and needle. Each tube was labelled by using codes describing the specific animal and flock/farm referring to its specific questionnaire for risk factors assessment. Due to some difficulties during sample handling and transportation from the fields in South Kivu up to Tanzania (SUA) in the laboratory blood samples were transported on a filter paper /FTA classic card (Whatman ®) for molecular analysis with a coding system referring to the questionnaire administrated.

3.6 Sample analysis

3.6.1 Serological analysis

The sera were analyzed using competitive enzyme linked immunosorbant assay (cELISA) kit (BDSL, Institute for Animal Heath, Pirbright, UK), according to Anderson *et al.* (1991). The procedure for performing cELISA is described in

Appendix 2. Briefly the plates were coated with PPRV antigen and incubated with test sera, control sera and monoclonal antibody. Afterwards, horseradish peroxidase conjugated rabbit anti-mouse immunoglobulin was added followed by substrate and chromogen. Colour was allowed to develop for 30 minutes followed by reading of plates at 492 nm (MTX Lab Systems, Vienna, USA). The ELISA micro-plates were read using ELISA DATA Interchange (EDI) software to give optical density (OD) values and percentage inhibition (PI) values were calculated using the following formula:

$$PI = 100 - \left(\frac{Replicate\ \ OD\ of\ each\ Control}{Median\ \ OD\ of\ Cm} \times 100\right).$$

The formula for the percentage inhibition (PI) which was used for acceptance of replicate values for test sera and diagnostic interpretation was:

$$PI=100 - \left(\frac{Replicate\ \ OD\ of\ each\ Test\ serum}{Median\ \ OD\ of\ Cm} \times 100\right).$$

Samples with a PI value of ≥50% were considered positive for PPR antibodies and test sera demonstrating mean PI value less than 50% were considered to be negative at CI 95% level for the prevalence of antibodies to PPRV.

3.6.2 Molecular analysis

3.6.2.1 RNA extraction

Viral RNA was recovered from the homogenized blood samples collected on the filter papers using QIAamp Viral RNA Mini extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions (Appendix 3). Samples were cut on filter paper with sterile scalped blade, lysed using a lysis buffer followed with protein precipitation using ethanol. The lysate was then passed through a Qiagen

column followed by washing and cleaning of bound RNA. Afterwards, the RNA was eluted with RNase free water. Extracted viral RNA was stored at -80ºC until amplification.

3.6.2.2 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 as shown in Table 1, was prepared using the AgPath-ID one-step RT-PCR kit (Applied Biosystems, Courtaboeuf, France) with PPRV specific primers (Eurogentec, Liège, Belgium) (Table 2). All reactions were run with Nigeria 75/1 vaccine strain as a positive control and nuclease-free water as the negative control.

Table 1: Reverse transcription polymerase chain reaction master mix components

No.	Component	Volume (µl)
1.	2X RT-PCR Buffer	12.5
2.	10µM Forward primer	1.0
3.	10µM Reverse primer	1.0
4.	Nuclease-free water	9.0
5.	25X RT-PCR Enzyme Mix	0.5
6.	Extracted RNA template	1.0
	Total volume per reaction	**25.0**

Table 2: The different primers used in the study

No.	Name	Gene	Location	Sequence	Amplicon Size	Reference
1.	NP3-forward	Nucleoprotein	1232-1255	5'- TCTCGGAAATCGCCTCACAGACTG -3'	351bp	Ularamu *et al*., 2012; Couacy-Hymann *et al*, 2002
2.	NP4-reverse	Nucleoprotein	1583-1560	5'- CCTCCTCCTGGTCCTCCAGAATCT -3'	351bp	
3.	PPRV-F-forward	Fusion protein	777-801	5'- GAGACTGAGTTTGTGACCTACAAGC -3'	322bp	Esmaelizad *et al*., 2011; Forsyth and Barrett, 1995
4.	PPRV-F-reverse	Fusion protein	1124-1148	5'- ATCACAGTGTTAAAGCCTGTAGAGG -3'	322bp	

The conditions of cycling for the NP3/NP4 primers were as described by Ularamu *et al*. (2012) (Fig. 8a) and for the F-gene refer to Esmaelizad *et al*. (2011) (Fig. 8b).

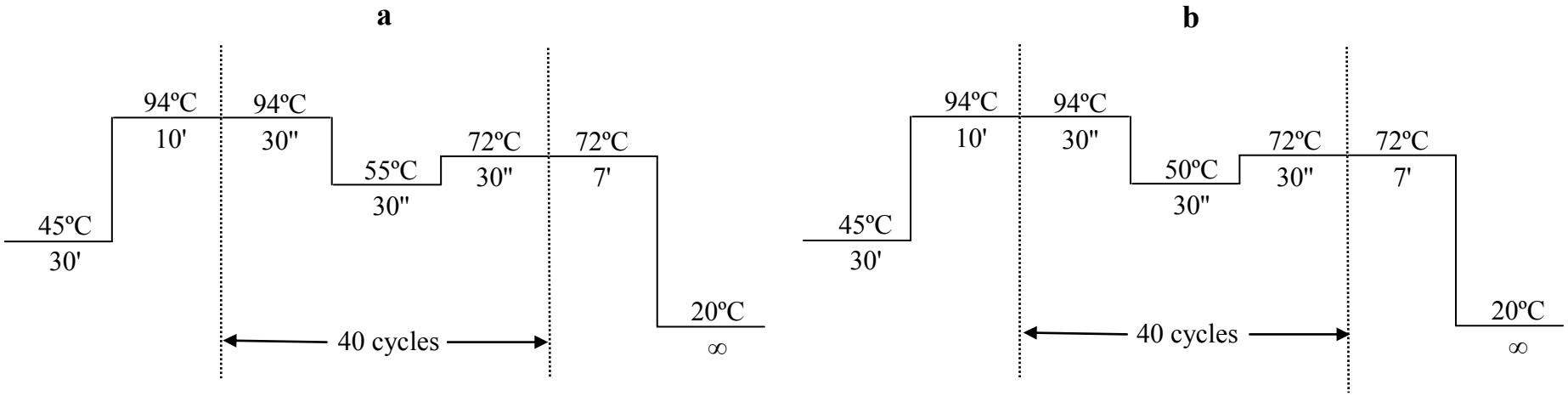

Figure 8: RT-PCR cycling conditions for the amplification of (a) N gene using NP3/NP4 primers and (b) F gene using PPRV-F-f/PPRV-F-r primers where ' represents minutes and " represents seconds and ∞ represents infinity.

3.6.3.3 Gel electrophoresis and visualization

Reverse transcriptase polymerase chain reaction products were separated by electrophoresis on a 1.5% agarose gel in 0.5% TAE buffer (SERVA Electrophoresis, Heidelberg, Germany) stained with 2 µl of GelRed nucleic acid stain (Phenix Research Products, Candler, USA). Each well was loaded with 5 µl of the PCR product and 1 µl of blue/orange 6X DNA loading dye (Promega, Madison, USA). Samples were separated along with a 100bp DNA ladder (Promega, Madison, USA) at 100 volts for 30 minutes. The agarose gel was visualized by ultraviolet fluorescence light using a gel documentation system (Biorad, Gel Doc ^{TM} EZ Imager).

3.7 Data collection for PPR risks factors

Laboratory results were completed with the risks factors found using structured questionnaire for every sample herd (Appendix 1). The significant associated factors, found from univariate analysis using chi-square were further analyzed multivariably by logistic regression.

3.8 Data Analysis

Epi info and Microsoft Office Excel 2010 were used to calculate frequencies of PPR samples prevalence. Chi-Square test (X^2) was used to test the significance of proportions between animals tested negative and those tested positive. F-test for multiple regressions (Logistic model) at 95% CI to see the correlation between the serological statues of animal with the risk factors associated with PPR transmission.

CHAPTER FOUR

4.0 RESULTS

4.1 Clinical Signs in Small Ruminants Suspected with PPR

Thepresent study was carried out in South-Kivu region of DRC in three territories including Shabunda, Mwenga and Fizi by visiting farms where there was a suspected outbreak of PPR. Some of the sick animals presented with clinical features reminiscent of PPR including erosions on the mucous membrane of the buccal cavity, ocular discharges, matting of eyelids and diarrhea. Apart from these clinical signs, some goats and sheep had nodules raised above the skin with an approximate diameter between 2 and 5cm (Fig.9).

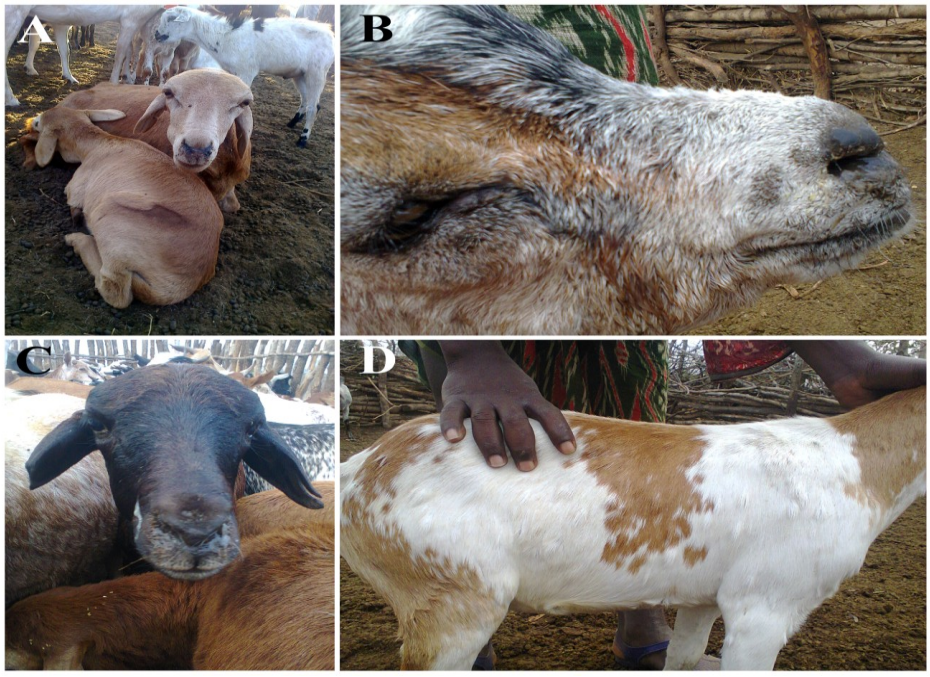

Figure 9: Clinical signs in goats and sheepsuspected with PPR. (A) Sheep and (B)goat with oculonasal discharges, (C)a goat with nasal erosion, (D)a goat withcutaneous nodules.

4.2 Prevalence ofantibodies against PPRV

The results showing seroprevalence of anti-PPRV antibodies according to territory, species and animal's age are shown in Table 3. A total of 319 sera samples from goats and sheep were collected from the three territories, where PPR outbreak has been previously reported in South-Kivu region. The overall PPR seropositivity in goats and sheep was 28.5%(n=319) out of which 11.3% (n=62) and 32.7% (n=257) seropositivity was found in sheep and goats, respectively.Considering different regions, the highest percentages of sero-positivity was found, respectively, in serum samples from Shabunda (34.5%) and Mwenga (29.4%) while the lowest sero-positivity was found in Fizi territory (19.4%). As mentioned above, the seroprevalence was also higher in goat (32.7%) than in sheep population (11.3%).

Table 3: The seroprevalence of antibodies against PPRV in small ruminants inShabunda, Mwenga and Fizi territories of South-Kivu region using cELISA.

Location		**PPR seroprevalence**		
	Species	**Total samples tested**	**cELISA positive samples**	**Seroprevalence (%)**
ShabundaTerritory	Goats	112	45	40.2
	Sheep	30	4	13.3
	Overall	**142**	**49**	**34.5**
Mwenga Territory	Goats	65	21	32.3
	Sheep	14	2	14.3
	Overall	**79**	**23**	**29.1**
Fizi Territory	Goats	80	18	22.5
	Sheep	18	1	5.6
	Overall	**98**	**19**	**19.4**
South-KivuProvince	Goats	257	84	32.7
	Sheep	62	7	11.3
	Overall	**319**	**91**	**28.5**

4.3 Risks factors associated with PPR transmission

The results on the presence of antibodies against PPRV were linked with the factors associated with PPRV transmission obtained after administering questionnaires to farmers. The results shown in Table 4, present the summary of the risk factors that are associated with the seroprevalence of PPR. All factors with p-value of less than 0.05 with a positive coefficient indicate the strong association between the risk factors for PPR transmission and the likelihood of finding antibodies against PPRV.

From the results indicated in Table 4 below that the lack of veterinary assistance for PPR diagnosis, control of the disease transmission and origin of animals that are kept in the different herds are not associated with the probability of animal to get a disease during any outbreak's occurrence. In contrast, grazing and farming systems, vaccination of small ruminants, age and sex of animal affect positively this likelihood (log likelihood = -21.706265; LR chi2 (9) = 21.24; Prob>chi2= 0.0116; pseudo R2= 0.3286).

Table 4: Logistic regression of factors associated with the presence of antibodies against PPRV in goats and sheep samples from South-Kivu in DRC

Variables	Coef.	Std. Err.	Z	p>|z|	[95% Conf. Interval]	
New animals introduction	-0.762191	1.021829	-0.75	0.456	-2.764939	1.240557
Grazing system	1.274841	1.078373	1.08	0.036	-3.388412	0.838731
Veterinary service	-0.427702	2.992279	-0.14	0.886	-6.292462	5.437057
Use of vaccine	2.090301	1.553719	1.35	0.179	-0.954932	5.135535
Bio safety	0.216319	1.628277	0.13	0.894	-2.975045	3.407684
Animal sex	2.187659	1.910057	1.15	0.252	-5.931302	1.555984
Animal age	0.106806	0.795910	0.34	0.041	-2.62802	0.491892
Animal origin	-1.276695	0.475360	-2.69	0.007	-2.208384	-0.345006
Farming system	0.264545	1.245253	0.21	0.022	-2.176105	2.705197
_Cons	7.337694	3.118044	2.35	0.019	1.22644	13.44895

Therefore, from the above Table 4 results, the age of a particular small ruminant (goats and/or sheep)can affect the probability of an animal to be a PPR positive or negative during any occurrence of outbreaks (Coef=0.1068064 and p-value<0.05). Animal aged of >12 months had a significantlyhigher seroprevalence are likely to be sero-positive compared to those aged of less than 12months in any outbreak occurrence. More seropositive cases were likely to be observed in animals kept in communal grazing system (coef. = 1.274841, p-value=0.036) and free-ranging farming system (Coef. =0.2645458, p-value=0.022) compare to the animals kept in zero-grazing farming system.

Table 5: Marginal effects of risks factors associated to PPR transmission in South-Kivu**.**

Variables	**Delta-methods**					
	dy/dx	**Std. Err.**	**Z**	**p>|z|**	**[95% Conf. Interval]**	
New animals introduction	-0.098052	0.129474	-0.75	0.449	-0.3518184	0.1557136
Grazing system	0.1640024	0.140512	1.08	0.036	-0.4394008	0.1113959
Veterinary service	-0.055022	0.380974	-0.14	0.885	-0.8017176	0.6916737
Use of vaccine	0.1389077	0.103016	1.34	0.185	-0.1289978	0.6668132
Bio safety measures	0.0278285	0.209707	0.13	0.894	-0.3831893	0.4388463
Animal sex	0.2814323	0.210349	1.17	0.242	-0.752508	0.1896433
Animal age	0.1374016	0.098386	0.35	0.041	-0.3302347	0.0554316
Animal origin	-0.164241	0.069411	-2.37	0.018	-0.3002842	-0.028197
Farming system	0.2140326	0.209713	0.21	0.021	-0.2789991	0.3470643

These results on marginal effects indicate the percentage of change of the probability for an animal to have or not have a PPRV during any outbreak occurrence in South-Kivu, when each variable change from one unit. Therefore, when the grazing system moves from zero-grazing to communal grazing system, the probability of an animal to be sero-positive increases of about 16.4%. The herd vaccination, the application of bio security measures at the farmer level to prevent PPR transmission, the change in sex of animal (male or female), animal physiological stage (age) and the farming system (free-ranging or control-ranging) increase respectively the percent of PPR sero-positivity of about 13.9 ; 2.8; 28.1; 13.7 and 21.4%. When the animal age

increases for one unit, its probability to be seropositive when there is an infection decreases ofabout 13.7%. Besides, when there is a new introduced animal in the herd and the absence of veterinary service in the region decrease this probability respectively of 9.5 and 5.5%. The origin of animals kept in the farms can also increase the decreases for 16.4% and camerathe probability of an animal to be sero-positive. However, the change in one unit of introduction of new animals from outside the herd decreases the probability of an animal to get infection if there is any outbreak occurrence for about 9.8%.

Table 6: Univariate analysis for risk factors associated with PPR seropositivity using Chi-square ($\chi 2$) test.

Risk factor	parameters	tested animals	+ve samples	Prevalence (%)	$\chi 2$	p-value
Animal age(month)	1-4	61	11	18.1	24.793	0.041*
	5-12	110	28	25.5		
	<12	148	52	35.1		
Grazing system	Communal	271	83	30.6	31.23	0.036*
	Zero-grazing	48	8	16.7		
Farming system	Free ranging	268	84	31.4	38.12	0.022*
	Zero-grazing	51	7	13.7		

From these findings, it can be speculated that the prevalence of PPR was affectedsignificantly with age, grazing system and farming (p-value>0.05) among thevisited territories in South-Kivu region. Sheep and goats of >12 months had a significantly higher seroprevalence, followed by animals with age from 5 to 12 months, while the low prevalence was found in kids aging 1 to 4 months. Animals

kept in communal grazing and free ranging are more affected by PPRV with respectively the seropositivity of 30.6% and 31.2% compare to animals kept in zero-grazing farming system (13.7%).

Table 7: Multiple regression analysis individual characteristics with odds ratios for infection with PPR

Variables	**Odds Ratio**	**Std. Err.**	**Z**	**p>|z|**	**[95% Conf. Interval]**	
New animals introduction	0.4666428	0.476829	-0.75	0.456	0.0629799	3.457538
Grazing system	6.2794755	0.301378	1.08	0.037	0.0337622	2.31343
Veterinary service	0.6520055	1.950982	-0.14	0.886	0.0018502	229.765
Use of vaccine	8.087353	12.56548	1.34	0.189	0.3848382	169.9552
Bio safety measures	1.241499	2.021504	0.13	0.894	0.0510452	30.19522
Animal sex	5.1121791	0.214268	1.17	0.243	0.002655	4.739747
Animal age	9.3436732	0.273533	0.35	0.041	0.0722213	1.635408
Animal origin	0.2789577	0.132605	-2.37	0.017	0.1098781	0.708216
Farming Systems	11.302839	1.622364	0.21	0.022	0.1134826	14.95726
_Cons	1537.163	4792.942	2.35	0.019	3.409071	693112.6

The odds ratio involves the relationship between the probability of success and failure. Thus, the higher the odds ratio, the higher is the probability of an event to happen. The introduction of new animals (small ruminants) kept in South-Kivu region is less likely to make an animal (goats and/or sheep) to be sero-positiveduring any infection (OR=0.46). Other variables such as presence of veterinary service in

the region to diagnose, control and prevent PPR outbreak occurrence and the origin of animal are likely to affect the PPR sero-prevalence in South-Kivu region respectively with OR=0.65 and OR=0.28, whereas the factors such as the grazing system, the usefulness of different vaccine, bio safety measures that is taken to prevent the disease at the farmer level, farming system and the age and sex of animals are more likely to influence the PPR sero-prevalence with respectively odds ratios of 6.28; 8.09; 1.24; 11.3; 9.34; 5.11. (Log likelihood = -21.706265, LR chi2 (9) = 21.24, Prob>chi2= 0.0116 and Pseudo R2= 0.3286).

4.4 Presence of PPRV in goats and sheep of South Kivu

Blood was collected and spotted onto FTA cards from a total of 65 small ruminants (14sheep and 51goats) in South-Kivu. RNA was extracted from FTA cards blood spots and screened for the presence of PPR RNA using primers that target the N and F genes (Table 2) using reverse transcription polymerase chain reaction (RT-PCR). No PPRV RNA was detected in any of the blood collected from sheep and goats from South-Kivu region, DRC.

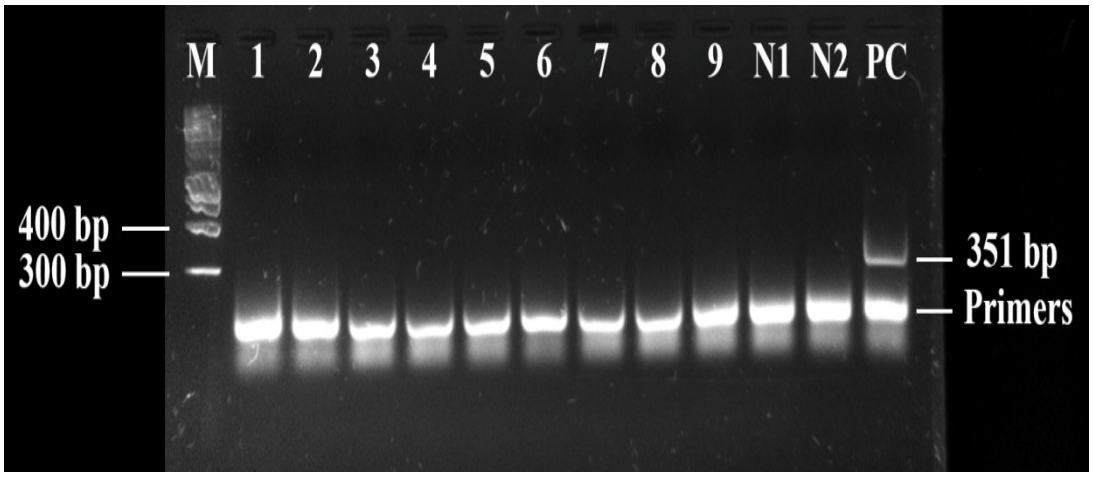

Figure 10: Detection of PPRV in goats and sheep of South Kivu South- Kivu.

A total of 9samples including six from goats (#1 through 6) and three from sheep (#7through 9) were included. A positive control (PC) from the vaccine strain of PPRV (Nig/75/1) was used as positive control and produced a base of 351 bp. No bands were observed in all samples tested (#1through 9) and the negative control from the extraction (N1) and for the master mix (N2).

CHAPTER FIVE

5.0 DISCUSSION

The present study was carried out in South-Kivu region of DRC in order to determine the seroprevalence and risk factors for *peste des petits ruminants* transmission.During this study, farms with small ruminants (goats and sheep) in South-Kivu region where there was a suspected outbreak of PPR were visited. PPR suspected goats and sheep presented with clinical signs including oculonasal discharges, diarrhea and cutaneous nodules. These clinical signs have been previously reported in sheep and goats confirmed with PPR (Ismail and House, 1990; Chauhan *et al.*, 2009; Zhao *et al.*, 2010; Muse *et al.,* 2012b; Kgotlele, *et al*., 2014).In the present study, lesions in goats were more severe than in sheep.Our results correlate with what was found by Muse *et al*. (2012b), who showed that goats were severely affected than sheep, which generally undergo a mild form of the disease.

Samples were collected from sheep and goats and tested for antibodies against PPRV using cELISA and presence of genome PPRV using RT-PCR(Anderson *et al.,* 1991). cELISAhas high specificity, sensitivity and can distinguish PPR from rinderpest (Sreenivasa *et al.*, 2006). Moreover, detection of PPRV specific antibodies in serum is the standard test for the rapid laboratory diagnosis of PPR.In the present study, cELISA was used to diagnose the presence of antibodies against PPRV in sera and estimate how widespread the infection was in South-Kivu regionwhile RT-PCR was used to detect the prevalence of PPRV infection. Seroprevalence data are useful because it gives information on the distribution of PPR in different geographical

areas and it can help in predicting the levelof protection in animals. This information is helpful in developing disease control strategies and understanding the patterns of PPRV infection. There are no known documented reports on previous vaccination in South-Kivu region where this study was conducted;hence presence of PPR antibodies can be directly attributed to natural PPR infection.The overall seroprevalence in small ruminants found in this study was28.5% (n=319) from which 11.3% (n=62) was found in sheep and 32.7 % (n=257) in goats (Table 3).Previous studies from northern and southern Tanzania done in 2011 and 2012 showed a seroprevalence of 45.4% (Swai *et al*., 2009) and 31.0% (Muse *et al*., 2012a). Different studies in Sudan have shown higher levels of seroprevalence including54% (Haroun *et al.*, 2002), 50.6% (Osman *et al.*, 2009), 62.8% (Saeed *et al.*, 2010) and 61.8% (Abdalla *et al.*2012).

Kayunze *et al.* (2012) affirmed that PPR is a transboundary animal disease (TAD) that has high mortality and potentialfor rapid spread across national borders or even across continents. Kaukarbayevich (2009) believed that PPR might have been introduced in African countries through the movement of live, infected animals. The differences in the PPRV seroprevalence found in neighboring countries compared to DRC could be also attributed to differences in management systems of small ruminants, sampling procedures used or technical knowledge levels of natural immunity and variable natural PPRV infection rates in different geographical areas as previously reported in other geographical areas bySingh *et al.*(2004).

Territories that registered higher seroprevalence of PPR were Shabunda (34.5%) and Mwenga (29.1%) whileFizi (19.4%) had the lowest PPR seroprevalence. The highest sero-positivity in Shabunda can be explained byintensive uncontrolled trans border small ruminants' movements between this territory with Kalima and Maniema where PPR outbreaks have been reported by FAO in 2012. In addition, poor management of animals and the use of communal grazing system could also contribute to this higher prevalence (Table 6 and appendix 4).

The interaction between sheep and goats in pastoralist system especially in the areas with high density of wild small ruminants like Shabunda and Mwenga could also have contributed to the higher PPR prevalence. The role of PPR transmission from wild to domestic ruminants has previously been reportedby Housawi et *al.*(2004) and Swai *et al.*(2009). In addition, Shabunda territory has big animal markets that allow contact of many animals from different areas and enhance viral transmission and spread. Domenech *et al*. (2006), described that trade of live animals at markets has been shown to be an important vehicle for transmission of infectious diseases. The highest PPR seroprevalence in Shabunda and Mwenga districts might be also related to the differences in climatic factors. These two territories recorded the highest annual rainfall, high humidity rate and wind-speed rates compared to Fizi territory.These findings are in line with the reports of Saeed *et al.* (2010)and Elnoman *et al.* (2011) proving that high rainfall and cool weather can contribute to PPR spreading therefore differed from the findings of Abdalla *et al.* (2012). Huyam *et al.* (2014) described that studies on risk factors are important for effective control and eradication of PPR. Besides climatic factors, seasonal and geographical variations influence PPR outbreak.

As shown in the results in Table 6, sheep and goats aged above 12 months hada significantlyhigher seroprevalence (35.1%) compared to those aged less than 12 months. As no any data is available in the DRC to compare with,these results can supportthe findings of Abubakar *et al.* (2009), who reported that highest PPR seroprevalence is likely to be seen in animals aged above 2 years old. Therefore, adult animals might be more vulnerable to PPR infections as compared to younger animals. However, Sarker and Islam (2011) reported the highest PPR seroprevalence in young animals in North Bengal because of poor immunity and nutrition.

Animals reared in communal system (pastoralist system or transhumance) and/or free ranging farming were likely to have high seroprevalence with respectiveseroprevalence levels of 30.6 and 31.3%, while low seroprevalence of 16.2% was found in the animals reared in zero grazing or intensive system (Table 6). These results were similar to the findings of Zahur *et al.* (2008) and Muse *et al.*(2012a), who found the source of the infection and spread of PPR wascontact between animals during communal grazing and housing.

Previous studies have shown that PPR samples could be spotted on FTA cards followed by successful detection of PPRV RNA by RT-PCR (Misinzo *et al*., 2015). In the present study, blood was obtained from sheep and goats and spotted on FTA cards which preserve genomic material and lyse the cells and viruses.After RNA extraction and RT-PCR using primers targeting the N and F genes, all samples were negative to PPRV molecular test using RT-PCR (Fig.10).This might be due to handling, the small amount of blood spotted onto FTA cards. In addition, it has been

reported that it is likely to find the PPRV in oculonasal discharges, swabs and tissues, rather than blood samples (Luka *et al.,* 2012). Banyard *et al*. (2010) found that PPR virus was secreted in tears, nasal discharge, secretions from coughing, and in the feces of infected animals.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Based on the results found in this study we can conclude that antibodies against *peste des petits ruminants* virusare widely prevalent in small ruminants (sheep and goats) keptin South-Kivu region located in DRC.ThePPRV antibodies could be detected by cELISA but no PPRV was found using RT-PCR targeting the N and F genes. Therefore, some clinical features reminiscent of PPR including oculonasal discharges, diarrhea and skin nodules were diagnosed in small ruminants kept is South-Kivu region of DRC. An overall seroprevalence for PPR of 28.5% was found and was mostly associated with some risks factors including, animal's age and sex, grazing and farming system. Communal grazing (open grazing)and free ranging farming systemswere most important factors associated with PPR transmission from an infected region to a none infected. On the other hand, PPR was mostly prevalent inanimals aged of >12 months. Goats were more affected than sheep.

6.2 Recommendations

Serology and molecular tools should be used in surveillance for PPR in order to detect carrier animals that do not show clinical signs. The animal transborder movementcontrol from infected area to noneinfected canreduce the spread of the disease over a large area. As the present survey provides the first preliminary information on PPRV seroprevalence in the Eastern part of DRC, more studies on the epidemiology of PPR are recommended to gather more data for effective control

measures including characterizing the strains that can be used for development of vaccines using local strains. Ministry of public health, veterinary service managements and other stakeholders of DRC should:

(i) Strengthen and implement the policies and guidelines to prevent and reduce transmission of PPR,

(ii) Conduct socio-economic study to assess the awareness of PPR in the community of DRC, and

(iii) Conduct further studies to determine the PPR genotype that is circulating in South-Kivu of DRC.

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APPENDICES

Appendix 1: Technical questionnaire

This questionnaire aims to identify people's knowledge and awareness of risk factors that could lead to PPRV transmission at the farm level.

1. Type of farming system?
    - Free ranging……/ - Not free ranking……

2. Have your animals (goats or sheep) experienced any of the following health problems?

Symptom	**Yes**	**No**	**Treatment provided**
Pneumonia			
Fever/ Salivation			
Diarrhea/ Digestive tracts			
Anorexia and			
Depression/nodules			
Nasal and ocular discharges			
difficult respiration			
Necrotic lesions on gum			
Erosion on the nasal mucosa reported			
Others (states)……			

3. Numbers of livestock keep in your farm?

Type of animal	**Local breed**		**GMO breed**	
	Male	**Female**	**Male**	**Female**
Goats				
Sheep				
Other (specify)				

GMO: Genetic modify organism

4. Characteristics of animal sampled

Parameters	**Type of animal**	
	Goats	**Sheep**
Sex(M/F)		
Age(month)		
Origin of animal		
weight(Kg)		

5. Geographic coordinate of your herd(location)

   Altitude (mm)……..……. Longitude...……..d/m/sec

6. What is the estimation of mortality rate in your herd per year for?

- Goats?.....percent / Sheep?.....percent

7. What is the estimation of morbidity rate in your herd per year for?

- Goats?.....percent / Sheep?.....percent

8. Have you introduce new animals in your herd? Yes…….No…….....

   If yes, what are their origins?

- Animal bought from live animal markets…….……....
- Animal bought from a far village/region/country location……...
- Animal bought from a nearest village/region/country location……...

9. Which type of grazing system do you apply in your herd?

- Communal grazing…….Housing…….zero grazing……..…..

10. Do have access on veterinary services in your village/herd? Yes…..No.……

11. Have you ever vaccinated your animal against PPRV? Yes……..No…….

12. Have you ever vaccinated against others viral diseases? Yes…….No……

Appendix 2: Comparative ELIZA procedure

Competitive ELISA (cELISA) is one of the most extensively used tests for serological screening and diagnosis of PPRV infected animals.

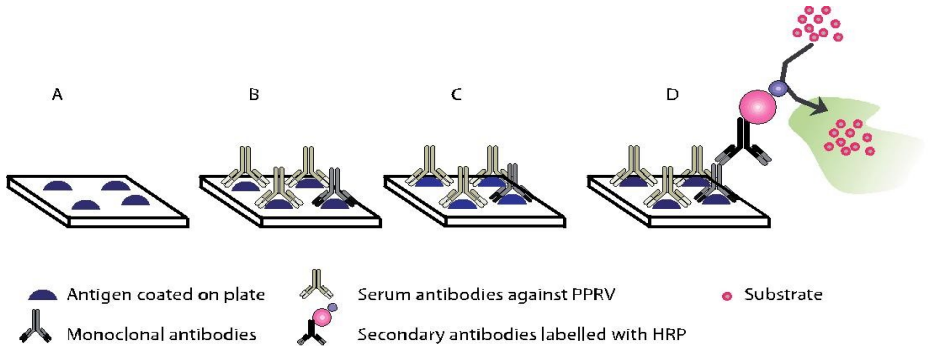

Figure 11:Principle of competitive ELISA assays. (A) Coating the antigen on a polystyrene plate. (B) Addition of serum sample and monoclonal antibodies rose against the N protein of PPRV. (C) Addition of secondary antibodies labeled with enzyme. (D) Addition of substrate and reading the plate.

A competitive ELISA based on the use of MAb anti-nucleocapsid protein and a recombinant nucleocapsid protein produced in the baculovirus has been described (Anderson *et al.,* 1991; Libeau *et al.*, 1995). Serodiagnosis of PPRV procedures:

1. Coat microtitre plates (e.g. high adsorption capacity NuncMaxisorb) with 50 µl of a predetermined dilution of N-PPR protein (produced by a recombinant baculovirus) for 1 hour at 37°C with constant agitation.
2. Wash the plates with washing solution (in the kit) three times and blot dry.
3. Distribute 45 µl of blocking buffer (PBS + 0.5% Tween 20 + 0.5 fetal calf serum) to all wells, and then add 5 µl of test sera to test wells (at a final dilution of 1/20) and 5 µl of the different control sera (strong positive, weak positive and negative serum) to the control wells.

4. Add 50 µl of MAb diluted 1/100 in blocking buffer, and incubate at 37°C for 1 h
5. Wash the plates three times and blot dry.
6. Add 50 µl of anti-mouse conjugate diluted 1/1000, and incubate at 37°C for 1 h.
7. Wash the plates three times and then prepare OPD in hydrogen peroxide solution. Add 50 µl of substrate/conjugate mixture to each well.
8. Stop the reaction after 10 minutes with 50 µl of 1 M sulphuric acid.
9. Read on an ELISA reader at 492 nm. The absorbance is converted to percentage inhibition (PI) using the formula: PI=100-Absorbance of the test wells/Absorbance of the MAb control wells ×100
10. Sera showing PI greater than 50% are positive.

Briefly:

- Prepare all buffers and reconstitute reagents as described in the above manual. Prepare working dilutions of reagents as just to relevant step.
- Dispense 50 µl of diluted of PPR antigen into all 96wells of micro plates ensuring even coverage of the bottom of each well. Cover microplates and incubate at 37°C with continuous shaking for 1 hour.
- Wash the microplates 3 times with wash buffer and immediately dispense 40 µl of blocking buffer to all 96wells of the microplates.
- According to the plate layout, add 10 µl volumes of test and control sera to the appropriate wells.
- Add 10 µl of blocking buffer to the monoclonal antibody control (Cm) wells
- Add 60 µl of blocking buffer to the conjugate control (Cc) wells.
- Add 50 µl of the working dilution of the monoclonal antibody to all the wells of

the microplate except the conjugate control (Cc) wells.

- Cover microplate and incubate at +37°C for 1 hour with continuous shaking.
- Wash the microplate 3 times with wash buffer.
- Add 50 µl of the working dilution of conjugate to all 96wells of the microplates ensuring even distribution over the bottom of each well.
- Cover microplates and incubate for 1 hour at +37°C with continuous shaking.
- Wash the microplates 3 times with wash buffer.
- Add 50 µl volumes of the substrate/chromogen solution to the wells of the microplates and incubate at room temperature for 10 minutes without plate shaking.
- Add 50 µl volumes of stopping solution to the wells of the microplates and read.

Appendix 3: RNA extraction and cDNA procedures

In a 1.5 ml microcentrifuge tube, 560 µl of prepared buffer AVL containing carrier RNA and 140 µl of either homogenized blood sample from FTA card was added. The contents were pulse vortexed for 15 seconds to ensure efficient lysis and incubated at room temperature for 10 minutes for complete viral particle lysis. Then the tube was centrifuged for 10 seconds at 6000 x g to remove drops from the inside of the lid. 560 µl of absolute ethanol (96–100%) was added to the sample mixture, then mixed by pulse-vortexing for 15 seconds and centrifuged for 10 seconds at 6000 x g to remove drops. After centrifugation, 630 µl of the sample mixture were transferred to the QIAamp mini column placed in a 2 ml collection tube. The column was centrifuged at 6000 x g for one minute. The collection tube with the filtrate was discarded and replaced with a clean 2 ml collection tube. The final sample mixture was transferred to the column, centrifuged at 6000 x g for one minute after which the collection tube with filtrate is discarded and replaced with a clean 2 ml collection tube. The bound nucleic acid was washed by adding 500 µl of buffer AW1 to the column and centrifuging at 6000 x g for one minute. The collection tube with the filtrate was discarded and replaced with a clean collection tube. The second wash, added 500 µl of buffer AW2 and centrifuged at 17 000 x g for three minutes. The collection tube with the filtrate was discarded and replaced with a clean 1.5 ml microcentrifuge tube. RNA was eluted by adding 60 µl of buffer AVE equilibrated to room temperature to the column. Colum contents were incubated at room temperature for one minute, and then centrifuged at 6000 x g for one minute. The filtrate, viral RNA, was immediately stored at -80°C until amplification.

Appendix 4: The presence of antibodies against PPRV in different farming systems, species, animal ages and sex in Shabunda, Mwenga and Fizi territories in South-Kivu

Samples	Territory	Species	Animal sex	Animal age (months)	Farming system	cELISA results
1	Shabunda	Goat	Male	1 to 4	Communal	+
2	Shabunda	Goat	Male	>12	Communal	+
3	Shabunda	Goat	Male	>12	Communal	-
4	Shabunda	Goat	Male	1 to 4	zero grazing	-
5	Shabunda	Goat	Male	1 to 4	zero grazing	-
6	Shabunda	Goat	Male	5 to 12	Communal	-
7	Shabunda	Goat	Male	>12	Communal	-
8	Shabunda	Goat	Male	1 to 4	zero grazing	-
9	Shabunda	Goat	Male	>12	Communal	-
10	Shabunda	Goat	Male	>12	zero grazing	-
11	Shabunda	Goat	Male	5 to 12	Communal	-
12	Shabunda	Goat	Male	>12	Communal	-
13	Shabunda	Goat	Female	1 to 4	Communal	+
14	Shabunda	Goat	Female	>12	zero grazing	+
15	Shabunda	Goat	Female	5 to 12	Communal	+
16	Shabunda	Goat	Female	>12	Communal	+
17	Shabunda	Goat	Female	>12	Communal	+
18	Shabunda	Goat	Female	5 to 12	Communal	+
19	Shabunda	Goat	Female	>12	zero grazing	+
20	Shabunda	Goat	Female	>12	Communal	+
21	Shabunda	Goat	Female	>12	Communal	+
22	Shabunda	Goat	Female	1 to 4	Communal	+
23	Shabunda	Goat	Female	>12	Communal	+

24	Shabunda	Goat	Female	>12	Communal	+
25	Shabunda	Goat	Female	>12	Communal	+
26	Shabunda	Goat	Female	>12	zero grazing	+
27	Shabunda	Goat	Female	5 to 12	Communal	+
28	Shabunda	Goat	Female	>12	Communal	+
29	Shabunda	Goat	Female	>12	Communal	+
30	Shabunda	Goat	Female	5 to 12	Communal	+
31	Shabunda	Goat	Female	>12	Communal	+
32	Shabunda	Goat	Female	>12	Communal	+
33	Shabunda	Goat	Female	>12	Communal	+
34	Shabunda	Goat	Female	5 to 12	Communal	+
35	Shabunda	Goat	Female	>12	Communal	+
36	Shabunda	Goat	Female	>12	Communal	+
37	Shabunda	Goat	Female	>12	Communal	+
38	Shabunda	Goat	Female	>12	zero grazing	+
39	Shabunda	Goat	Female	5 to 12	Communal	+
40	Shabunda	Goat	Female	>12	Communal	+
41	Shabunda	Goat	Female	>12	Communal	+
42	Shabunda	Goat	Female	1 to 4	Communal	+
43	Shabunda	Goat	Female	>12	Communal	+
44	Shabunda	Goat	Female	>12	Communal	+
45	Shabunda	Goat	Female	5 to 12	zero grazing	+
46	Shabunda	Goat	Female	>12	Communal	+
47	Shabunda	Goat	Female	>12	Communal	+
48	Shabunda	Goat	Female	5 to 12	Communal	+
49	Shabunda	Goat	Female	>12	Communal	+
50	Shabunda	Goat	Female	>12	Communal	+
51	Shabunda	Goat	Female	1 to 4	Communal	+

52	Shabunda	Goat	Female	>12	Communal	+
53	Shabunda	Goat	Female	5 to 12	Communal	+
54	Shabunda	Goat	Female	5 to 12	Communal	+
55	Shabunda	Goat	Female	>12	Communal	+
56	Shabunda	Goat	Female	1 to 4	zero grazing	-
57	Shabunda	Goat	Female	1 to 4	zero grazing	-
58	Shabunda	Goat	Female	1 to 4	zero grazing	-
59	Shabunda	Goat	Female	5 to 12	zero grazing	-
60	Shabunda	Goat	Female	5 to 12	zero grazing	-
61	Shabunda	Goat	Female	5 to 12	zero grazing	-
62	Shabunda	Goat	Female	5 to 12	zero grazing	-
63	Shabunda	Goat	Female	5 to 12	zero grazing	-
64	Shabunda	Goat	Female	5 to 12	Communal	-
65	Shabunda	Goat	Female	5 to 12	Communal	-
66	Shabunda	Goat	Female	5 to 12	Communal	-
67	Shabunda	Goat	Female	5 to 12	Communal	-
68	Shabunda	Goat	Female	>12	Communal	-
69	Shabunda	Goat	Female	1 to 4	Communal	-
70	Shabunda	Goat	Female	1 to 4	Communal	-
71	Shabunda	Goat	Female	>12	Communal	-
72	Shabunda	Goat	Female	5 to 12	Communal	-
73	Shabunda	Goat	Female	>12	Communal	-
74	Shabunda	Goat	Female	1 to 4	Communal	-
75	Shabunda	Goat	Female	>12	Communal	-
76	Shabunda	Goat	Female	5 to 12	Communal	-
77	Shabunda	Goat	Female	5 to 12	zero grazing	-
78	Shabunda	Goat	Female	>12	Communal	-
79	Shabunda	Goat	Female	>12	Communal	-

80	Shabunda	Goat	Female	1 to 4	Communal	-
81	Shabunda	Goat	Female	1 to 4	zero grazing	-
82	Shabunda	Goat	Female	>12	Communal	-
83	Shabunda	Goat	Female	5 to 12	Communal	-
84	Shabunda	Goat	Female	5 to 12	Communal	-
85	Shabunda	Goat	Female	5 to 12	Communal	-
86	Shabunda	Goat	Female	5 to 12	Communal	-
87	Shabunda	Goat	Female	5 to 12	Communal	-
88	Shabunda	Goat	Female	5 to 12	Communal	-
89	Shabunda	Goat	Female	5 to 12	Communal	-
90	Shabunda	Goat	Female	5 to 12	Communal	-
91	Shabunda	Goat	Female	5 to 12	Communal	-
92	Shabunda	Goat	Female	>12	Communal	-
93	Shabunda	Goat	Female	>12	Communal	-
94	Shabunda	Goat	Female	1 to 4	Communal	-
95	Shabunda	Goat	Female	1 to 4	zero grazing	-
96	Shabunda	Goat	Female	1 to 4	Communal	-
97	Shabunda	Goat	Female	1 to 4	zero grazing	-
98	Shabunda	Goat	Female	>12	Communal	-
99	Shabunda	Goat	Female	5 to 12	Communal	-
100	Shabunda	Goat	Female	>12	Communal	-
101	Shabunda	Goat	Female	1 to 4	Communal	-
102	Shabunda	Goat	Female	1 to 4	Communal	-
103	Shabunda	Goat	Female	>12	Communal	-
104	Shabunda	Goat	Female	>12	Communal	-
105	Shabunda	Goat	Female	5 to 12	Communal	-
106	Shabunda	Goat	Female	5 to 12	Communal	-
107	Shabunda	Goat	Female	5 to 12	Communal	-

108	Shabunda	Goat	Female	5 to 12	Communal	-
109	Shabunda	Goat	Female	5 to 12	Communal	-
110	Shabunda	Goat	Female	5 to 12	Communal	-
111	Shabunda	Goat	Female	5 to 12	zero grazing	-
112	Shabunda	Goat	Female	>12	Communal	-
113	Shabunda	Sheep	Male	5 to 12	Communal	+
114	Shabunda	Sheep	Male	>12	Communal	-
115	Shabunda	Sheep	Male	5 to 12	Communal	-
116	Shabunda	Sheep	Male	1 to 4	zero grazing	-
117	Shabunda	Sheep	Male	1 to 4	Communal	-
118	Shabunda	Sheep	Male	>12	Communal	-
119	Shabunda	Sheep	Male	>12	Communal	-
120	Shabunda	Sheep	Female	5 to 12	Communal	-
121	Shabunda	Sheep	Female	>12	Communal	-
122	Shabunda	Sheep	Female	>12	Communal	-
123	Shabunda	Sheep	Female	5 to 12	zero grazing	-
124	Shabunda	Sheep	Female	>12	Communal	-
125	Shabunda	Sheep	Female	>12	zero grazing	-
126	Shabunda	Sheep	Female	>12	Communal	-
127	Shabunda	Sheep	Female	5 to 12	Communal	+
128	Shabunda	Sheep	Female	1 to 4	Communal	+
129	Shabunda	Sheep	Female	>12	Communal	+
130	Shabunda	Sheep	Female	>12	Communal	-
131	Shabunda	Sheep	Female	>12	Communal	-
132	Shabunda	Sheep	Female	>12	Communal	-
133	Shabunda	Sheep	Female	5 to 12	Communal	-
134	Shabunda	Sheep	Female	>12	Communal	-
135	Shabunda	Sheep	Female	>12	Communal	-

136	Shabunda	Sheep	Female	>12	Communal	-
137	Shabunda	Sheep	Female	5 to 12	Communal	-
138	Shabunda	Sheep	Female	>12	Communal	-
139	Shabunda	Sheep	Female	>12	Communal	-
140	Shabunda	Sheep	Female	>12	Communal	-
141	Shabunda	Sheep	Female	1 to 4	Communal	-
142	Shabunda	Sheep	Female	>12	Communal	-
143	Mwenga	Goat	Male	>12	Communal	-
144	Mwenga	Goat	Male	5 to 12	zero grazing	-
145	Mwenga	Goat	Male	5 to 12	Communal	-
146	Mwenga	Goat	Male	>12	zero grazing	-
147	Mwenga	Goat	Male	5 to 12	Communal	+
148	Mwenga	Goat	Male	>12	Communal	-
149	Mwenga	Goat	Female	>12	Communal	+
150	Mwenga	Goat	Female	1 to 4	Communal	+
151	Mwenga	Goat	Female	>12	zero grazing	+
152	Mwenga	Goat	Female	5 to 12	Communal	+
153	Mwenga	Goat	Female	>12	Communal	+
154	Mwenga	Goat	Female	5 to 12	Communal	+
155	Mwenga	Goat	Female	>12	Communal	+
156	Mwenga	Goat	Female	1 to 4	Communal	+
157	Mwenga	Goat	Female	>12	Communal	+
158	Mwenga	Goat	Female	>12	Communal	+
159	Mwenga	Goat	Female	5 to 12	Communal	+
160	Mwenga	Goat	Female	>12	Communal	+
161	Mwenga	Goat	Female	>12	Communal	+
162	Mwenga	Goat	Female	5 to 12	Communal	+
163	Mwenga	Goat	Female	>12	Communal	+

164	Mwenga	Goat	Female	>12	Communal	+
165	Mwenga	Goat	Female	1 to 4	Communal	+
166	Mwenga	Goat	Female	>12	Communal	+
167	Mwenga	Goat	Female	5 to 12	Communal	+
168	Mwenga	Goat	Female	1 to 4	Communal	+
169	Mwenga	Goat	Female	5 to 12	zero grazing	-
170	Mwenga	Goat	Female	5 to 12	zero grazing	-
171	Mwenga	Goat	Female	5 to 12	zero grazing	-
172	Mwenga	Goat	Female	1 to 4	zero grazing	-
173	Mwenga	Goat	Female	1 to 4	zero grazing	-
174	Mwenga	Goat	Female	1 to 4	zero grazing	-
175	Mwenga	Goat	Female	1 to 4	zero grazing	-
176	Mwenga	Goat	Female	1 to 4	zero grazing	-
177	Mwenga	Goat	Female	1 to 4	zero grazing	-
178	Mwenga	Goat	Female	1 to 4	zero grazing	-
179	Mwenga	Goat	Female	1 to 4	Communal	-
180	Mwenga	Goat	Female	>12	Communal	-
181	Mwenga	Goat	Female	5 to 12	Communal	-
182	Mwenga	Goat	Female	5 to 12	Communal	-
183	Mwenga	Goat	Female	5 to 12	Communal	-
184	Mwenga	Goat	Female	5 to 12	Communal	-
185	Mwenga	Goat	Female	5 to 12	Communal	-
186	Mwenga	Goat	Female	5 to 12	Communal	-
187	Mwenga	Goat	Female	5 to 12	Communal	-
188	Mwenga	Goat	Female	5 to 12	Communal	-
189	Mwenga	Goat	Female	>12	Communal	-
190	Mwenga	Goat	Female	>12	Communal	-
191	Mwenga	Goat	Female	>12	Communal	-

192	Mwenga	Goat	Female	1 to 4	Communal	-
193	Mwenga	Goat	Female	1 to 4	Communal	-
194	Mwenga	Goat	Female	1 to 4	Communal	-
195	Mwenga	Goat	Female	1 to 4	Communal	-
196	Mwenga	Goat	Female	1 to 4	Communal	-
197	Mwenga	Goat	Female	>12	Communal	-
198	Mwenga	Goat	Female	5 to 12	Communal	-
199	Mwenga	Goat	Female	>12	Communal	-
200	Mwenga	Goat	Female	5 to 12	Communal	-
201	Mwenga	Goat	Female	>12	Communal	-
202	Mwenga	Goat	Female	5 to 12	Communal	-
203	Mwenga	Goat	Female	>12	Communal	-
204	Mwenga	Goat	Female	>12	Communal	-
205	Mwenga	Goat	Female	>12	Communal	-
206	Mwenga	Goat	Female	>12	Communal	-
207	Mwenga	Goat	Female	5 to 12	Communal	-
208	Mwenga	Sheep	Male	>12	Communal	-
209	Mwenga	Sheep	Male	>12	zero grazing	-
210	Mwenga	Sheep	Male	>12	Communal	-
211	Mwenga	Sheep	Female	5 to 12	Communal	-
212	Mwenga	Sheep	Female	>12	Communal	-
213	Mwenga	Sheep	Female	>12	Communal	-
214	Mwenga	Sheep	Female	5 to 12	Communal	+
215	Mwenga	Sheep	Female	1 to 4	Communal	+
216	Mwenga	Sheep	Female	>12	Communal	-
217	Mwenga	Sheep	Female	>12	zero grazing	-
218	Mwenga	Sheep	Female	5 to 12	zero grazing	-
219	Mwenga	Sheep	Female	5 to 12	Communal	-

220	Mwenga	Sheep	Female	>12	Communal	-
221	Mwenga	Sheep	Female	>12	Communal	-
222	Fizi	Goat	Male	>12	Communal	-
223	Fizi	Goat	Male	>12	Communal	-
224	Fizi	Goat	Male	>12	Communal	+
225	Fizi	Goat	Male	>12	Communal	-
226	Fizi	Goat	Male	>12	Communal	+
227	Fizi	Goat	Male	>12	Communal	-
228	Fizi	Goat	Male	>12	Communal	-
229	Fizi	Goat	Male	>12	Communal	-
230	Fizi	Goat	Male	>12	Communal	-
231	Fizi	Goat	Female	>12	Communal	+
232	Fizi	Goat	Female	>12	Communal	+
233	Fizi	Goat	Female	5 to 12	Communal	+
234	Fizi	Goat	Female	5 to 12	Communal	+
235	Fizi	Goat	Female	5 to 12	Communal	+
236	Fizi	Goat	Female	>12	zero grazing	+
237	Fizi	Goat	Female	>12	Communal	+
238	Fizi	Goat	Female	5 to 12	Communal	+
239	Fizi	Goat	Female	>12	zero grazing	+
240	Fizi	Goat	Female	>12	Communal	+
241	Fizi	Goat	Female	5 to 12	Communal	+
242	Fizi	Goat	Female	>12	Communal	+
243	Fizi	Goat	Female	>12	Communal	+
244	Fizi	Goat	Female	5 to 12	Communal	+
245	Fizi	Goat	Female	5 to 12	Communal	+
246	Fizi	Goat	Female	>12	Communal	+
247	Fizi	Goat	Female	>12	zero grazing	-

248	Fizi	Goat	Female	>12	Communal	-
249	Fizi	Goat	Female	1 to 4	Communal	-
250	Fizi	Goat	Female	1 to 4	zero grazing	-
251	Fizi	Goat	Female	1 to 4	Communal	-
252	Fizi	Goat	Female	1 to 4	zero grazing	-
253	Fizi	Goat	Female	1 to 4	Communal	-
254	Fizi	Goat	Female	1 to 4	zero grazing	-
255	Fizi	Goat	Female	1 to 4	Communal	-
256	Fizi	Goat	Female	1 to 4	Communal	-
257	Fizi	Goat	Female	1 to 4	zero grazing	-
258	Fizi	Goat	Female	1 to 4	Communal	-
259	Fizi	Goat	Female	1 to 4	Communal	-
260	Fizi	Goat	Female	1 to 4	Communal	-
261	Fizi	Goat	Female	>12	Communal	-
262	Fizi	Goat	Female	>12	zero grazing	-
263	Fizi	Goat	Female	5 to 12	Communal	-
264	Fizi	Goat	Female	5 to 12	Communal	-
265	Fizi	Goat	Female	5 to 12	Communal	-
266	Fizi	Goat	Female	5 to 12	Communal	-
267	Fizi	Goat	Female	5 to 12	Communal	-
268	Fizi	Goat	Female	5 to 12	Communal	-
269	Fizi	Goat	Female	>12	zero grazing	-
270	Fizi	Goat	Female	5 to 12	Communal	-
271	Fizi	Goat	Female	>12	Communal	-
272	Fizi	Goat	Female	5 to 12	zero grazing	-
273	Fizi	Goat	Female	>12	Communal	-
274	Fizi	Goat	Female	>12	Communal	-
275	Fizi	Goat	Female	1 to 4	Communal	-

276	Fizi	Goat	Female	1 to 4	zero grazing	-
277	Fizi	Goat	Female	>12	Communal	-
278	Fizi	Goat	Female	>12	Communal	-
279	Fizi	Goat	Female	5 to 12	Communal	-
280	Fizi	Goat	Female	>12	Communal	-
281	Fizi	Goat	Female	5 to 12	Communal	-
282	Fizi	Goat	Female	>12	Communal	-
283	Fizi	Goat	Female	>12	Communal	-
284	Fizi	Goat	Female	5 to 12	Communal	-
285	Fizi	Goat	Female	>12	Communal	-
286	Fizi	Goat	Female	5 to 12	zero grazing	-
287	Fizi	Goat	Female	>12	Communal	-
288	Fizi	Goat	Female	5 to 12	Communal	-
289	Fizi	Goat	Female	>12	Communal	-
290	Fizi	Goat	Female	>12	Communal	-
291	Fizi	Goat	Female	5 to 12	Communal	-
292	Fizi	Goat	Female	>12	Communal	-
293	Fizi	Goat	Female	>12	Communal	-
294	Fizi	Goat	Female	5 to 12	Communal	-
295	Fizi	Goat	Female	5 to 12	Communal	-
296	Fizi	Goat	Female	>12	Communal	-
297	Fizi	Goat	Female	5 to 12	Communal	-
298	Fizi	Goat	Female	>12	Communal	-
299	Fizi	Goat	Female	>12	Communal	-
300	Fizi	Goat	Female	5 to 12	Communal	-
301	Fizi	Goat	Female	5 to 12	Communal	-
302	Fizi	Sheep	Male	>12	Communal	-
303	Fizi	Sheep	Male	>12	Communal	-

304	Fizi	Sheep	Male	5 to 12	zero grazing	-
305	Fizi	Sheep	Male	>12	Communal	-
306	Fizi	Sheep	Female	5 to 12	Communal	+
307	Fizi	Sheep	Female	>12	Communal	-
308	Fizi	Sheep	Female	5 to 12	Communal	-
309	Fizi	Sheep	Female	>12	zero grazing	-
310	Fizi	Sheep	Female	1 to 4	Communal	-
311	Fizi	Sheep	Female	1 to 4	zero grazing	-
312	Fizi	Sheep	Female	1 to 4	Communal	-
313	Fizi	Sheep	Female	>12	Communal	-
314	Fizi	Sheep	Female	5 to 12	Communal	-
315	Fizi	Sheep	Female	5 to 12	Communal	-
316	Fizi	Sheep	Female	5 to 12	Communal	-
317	Fizi	Sheep	Female	5 to 12	Communal	-
318	Fizi	Sheep	Female	>12	Communal	-
319	Fizi	Sheep	Female	5 to 12	Communal	-