EPIDEMIOLOGY OF PESTE DES PETITS RUMINANTS IN RELATION TO SMALL RUMINANTS MOVEMENTS AND INTERACTIONS WITH WILDLIFE IN TANZANIA

DANIEL PIUS MDETELE

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

2021

EXTENDED ABSTRACT

Peste des petits ruminants (PPR) is a highly contagious transboundary animal disease of domestic small ruminants, camels and some wild artiodactyls. It is caused by *Small ruminant morbillivirus* (PPRV) of the family *Paramyxoviridae* classified into four genetically distinct lineages I, II, III and IV; and shares genetic and antigenic characteristics with rinderpest (RP) virus. The disease has significant socio-economic impact on communities which largely depend on livestock for livelihood, and is a threat to endangered susceptible wild species. This study aimed at investigating the spread and maintenance of PPR in the pastoral and agropastoral communities characterized by extensive mobility, in some areas interacting with wildlife, in order to generate necessary information for proper planning of control and eradication strategies for PPR. A Cross sectional studies, complimented with review of previous studies were carried out during the present study. Data collected from livestock and wildlife populations, samples collected from different agro ecological zones and wildlife from different habitats were involved in the study. In ecological studies, purposive sampling was performed in sheep and goats from 32 districts where PPR surveillance had never been carried out after the confirmation of PPR in Tanzania. The 32 districts involved in the study, included 3 (9.4%), 12 (37.5%) and 17 (53.1%) districts from the coast, semi-arid and plateau ecological zones, respectively. For epidemiological characterization of PPR, a total of 78 flocks of sheep and goats were investigated from Karatu (n=10), Longido (n=9), Meatu (n=7), Monduli (n=11), Ngorongoro Conservation Area Authority (NCAA) (n=13), Ngorongoro (n=18) and Serengeti (n=10).

For the investigation of PPR seroprevalence in wildlife from different habitats, four species of wildlife; buffalos (*Syncerus caffer*), impalas (*Aepyceros melampus*), Grant's gazelles (*Nanger granti*) and Thomson's gazelles (*Eudorcas thomsonii*) were sampled. Sample sizes were calculated based on wildlife population obtained from TAWIRI aerial census of 2009 and 2010. PROMESA software (http://www.promesa.co .nz/ProMESA.htm) was used to estimate sample size per location in three types of wildlife habitats. Habitats were selected based on the level of contacts with wildlife. The habitats included Serengeti National Park (SNP) - designated only for wildlife, Loliondo Game Controlled Area (LGCA) - designated for wildlife livestock and other human activities and Ngorongoro Conservation Area Authority (NCAA) - designated for wildlife and livestock only. Chemical immobilization technique was used to capture buffalos and impalas, whereas gazelles were captured by using a modified netting technique, with trap made using locally available materials. With this technique three vehicles were used to head the animals towards the trap. All collected samples from livestock and wildlife were analysed at the SACIDS laboratory of Sokoine University of Agriculture.

On ecological studies, the overall seropositivity across all agro ecological zones based on c-ELISA was 20.1%, of which 18.8%, 9.4%, 37.5% and 34.4% districts had very high, high, low and zero PPR seroprevalence respectively. Very high and high seroprevalence were frequently recorded in the semi-arid districts. Zero and low seroprevalence were mostly observed in districts from plateaux ecological zone. Statistically there were significant differences in PPR seroprevalences among districts of different ecological zones. On PPR outbreak characterization in the Serengeti ecosystem, a total of 160 samples were collected from clinically diagnosed cases, out of which 12 and 11 cases were confirmed using a lateral flow device (LFD) and real time reverse transcription polymerase chain reaction (qRT-PCR) tests, respectively. Of the confirmed cases about

60% of the animals were aged below six months of age with body temperature ranging from 38.5 to 41.3ºC, about 70% had lacrimation and only 45.5% had diarrhoea. Lineage III of PPRV was found to be circulating in the area. Semi structured interviews indicated pastoral communities were aware of PPR syndromes and had traditional names and remedies unlike in the agropastoral communities who mostly used Swahili terminologies.

There was no clinical case of PPR observed in the 3 different wildlife habitats. However, a cross sectional survey was conducted to determine the seroprevalence of PPR in wildlife species. A total of 270 wildlife were captured, 26 (9.6%) from LGCA, 75 (27.8%) from NCAA and 169 (62.6%) from SNP, out of which two (7.7%), seven (9.3%) and 30 (17.8%) were seropositive, respectively. Results for one (3.8%), six (8%) and 42 (24.8%) animals from LGCA, NCAA and SNP, respectively, were doubtful. There were no statistically significant differences in seropositivity between habitats, species, age and sex. A modified netting technique developed and used during the present study, showed high animal and operator safety levels with minimal injuries compared to previous techniques. With this technique it was possible to capture even flighty animals that behave nervously because of hunting and other human activities, including Thomson's gazelles (*Eudorcas thomsonii*), a species previously found to be difficult to capture by netting. Peste des petits ruminants was introduced in Tanzania before its confirmation in 2008 in northern Tanzania and has been spreading into different areas of the country through live animal trade and pastoralist migration. Seroprevalence of the disease in sheep and goats has been found to be higher in semi-arid agro-ecological zone. Peste des petits ruminants outbreak characterization in areas where livestock coexist with wildlife indicated age, temperature and lacrimation to be important components of the case definition for PPR syndromic diagnosis. Lineage III was found to be the lineage circulating at the moment in the area.

Although no clinical cases of PPR were observed in wildlife, PPR antibodies have been recovered in wildlife coexisting with livestock confirmed to have PPR cases which indicates that at one point wild animals contracted the virus. There was no statistically significant difference in the PPR seroprevalence between wildlife coexisting with livestock and those with no contact with livestock. Therefore, surveillance, prevention, control and eradication strategies for PPR should consider the agroecological zones favouring survival and perpetuation of the virus among reservoir hosts and the susceptible populations in these areas. Pastoral and live animal traders' movements need to be considered in planning and implementation of PPR control strategies. Veterinary services and conservation authorities are encouraged to work together on planning PPR surveillance and control at different levels. On syndromic diagnosis of PPR in endemic settings need to consider age, body temperature and lacrimation on case definition. For species other than sheep and goats the c-ELISA test kits need to be validated as there were higher levels of doubtful results on laboratory analysis in wildlife samples.

DECLARATION

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

_____ _____

Daniel Pius Mdetele Date

(PhD Candidate)

The above declaration is confirmed by;

_____ _____

Prof. Gerald Misinzo Date

(Supervisor)

_____ _____

Prof. Erick Komba Date

(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENTS

The present study which led to the production of this thesis received financial support from Global Challenge Research Funds (GCRF) PPR project. The project was implemented by Royal Veterinary College (RVC), Directorate of Veterinary Services - Tanzania, Tanzania Wildlife Research Institute (TAWIRI), SACIDS foundation for One Health, Ngorongoro Conservation Area Authority (NCAA), Tanzania National Park Authority (TANAPA), Tanzania Wildlife Authority (TAWA) and French Agricultural Research Centre for International Development (CIRAD). I am very grateful to all those institutions for their support which provided me with such a great opportunity to pursue my career. It is my pleasure to thank all my supervisors and mentors who guided me from research proposal formulation to the production of this thesis; Prof. Mark Rweyemamu, Prof. Gerald Misinzo, Prof. Esron Karimuribo, Prof. Erick Komba, Prof. Richard Kock, Dr. Bryony Jones and Prof. Satya Parida. The PhD journey was long and difficult, but with their dedicated moral and material support, this work has been completed. Gerald, Mark and Erick are sincerely thanked for always coming with alternative plans at critical times; Richard and Bryony were so supportive and encouraging during the whole period of field work and manuscript preparations. Prof. Satya, Dr. Mana Mahapatra and other laboratory staff (Mariam Makange, Anna Rogers and Vero Muhoja) are highly thanked for their support during laboratory work. I am deeply indebted to the Ministry of Livestock

and Fisheries, my employer for granting me a fully-supported study leave and permission to pursue my studies. On that behalf, let me sincerely thank Dr. Abdul Hayghaimo, Dr. Martin Ruheta and Prof. Hezron Nonga who at different times during the course of my study were Directors of Veterinary Services. Moreover, I extend a word of thanks to all colleagues from Directorate of Veterinary Services and TAWIRI. Finally, my sincere gratitude goes to my family: My wife Martha, our daughter Rosemary and our Sons Golden, William and Jacob, for their patience as they had to endure long period of my absence during my studies. I also appreciate the moral, material and psychological support from my relatives, neighbors and friends. Many people played individual or collective roles of one kind or the other, knowingly or unknowingly and it is impossible to mention all of them. I therefore extend my hand of appreciation to all of you.

DEDICATION

This work is dedicated to my parents the late Mr. Pius Mdetele and my mother Fedelika Mwena, for nurturing my early childhood talents that made me reach where I am today. As well, to my late wife Tecla Lucas Myumbilwa and my beloved family, my wife Martha and our children Rosemary, Golden, William and Jacob.

LIST OF PUBLICATIONS

1. **Mdetele D**, Komba E, Misago S, Kock R, Misinzo G and Jones B (2021) Review of Peste des Petits Ruminants occurrence and spread in Tanzania. *Animals* https://doi.org/10.3390/ani11061698.

2. **Mdetele D**, Seth M, Kabululu M, Misinzo G and Komba E (2020) A comparative study of the seroprevalences of peste des petits ruminants virus among districts of different agroecological zones in Tanzania. *East African Journal of Science, Technology and Innovation, Vol. 1 (3): 2020. DOI: 10.37425/eajsti.v1i3.167.*

3. **Mdetele D**, Komba E, Misago S, Kock R, Misinzo G and Jones B (2021) Characterization of peste des petits ruminants outbreaks in the Serengeti Ecosystem. (submittied to Animal Journal).

4. **Mdetele D**, Misinzo G, Komba, E, Shemanche J, Eblate E, Mdaki M, Keyyu J and Kock R (2020) Modified netting technique for capturing gazelles in Serengeti, Ngorongoro and Loliondo, Tanzania. *African Journal of Ecology 00;2020: 1-7. DOI:10.1111/aje.12813.*

5. **Mdetele D,** Mdaki M, Makange M, Elbret E, Shemanche J, Kabululu M, Misinzo G, Keyyu J, Komba E, Jones B and Kock R (2021) Comparative study of peste des petitis ruminants virus seroprevalence among wildlife from different habitats of the Serengeti ecosystem, Tanzania. (In preparations).

TABLE OF CONTENTS

LIST OF APPENDICES

LIST OF ABREVIATION AND SYMBOLS

ARIS	Animal Resource Information System
AU-IBAR	African Union - Inter African Bureau for Animal Resource
AU-PANVAC	African Union - Pan African Veterinary Vaccine Centre
CCPP	Contagious Caprine Pleuropneumonia
C-ELISA	Competitive Enzyme - Linked Immunosorbent Assay
CPE	Cytopathic Effect
CVMBS	College of Veterinary Medicine and Biomedical Sciences
DRC	Democratic Republic of the Congo
DVO	District Veterinary Officer
DVS	Directorate of Veterinary Services
EAC	East African Community
ECTAD	Emergency Centre for Transboundary Animal Diseases
ELISA	Enzyme - Linked Immunossorbent Assay
EU	European Union
FAO	Food and Agriculture Organization of the united Nations
FMD	Foot and Mouth Disease
GDP	Gross Development Product
GF-TADs	Global Framework for the Progressive Control of TADs
GHoA	Greater Horn of Africa
IGAD	Inter-Governmental Authority on Development
ILRI	International Livestock Research Institute
KWS	Kenya Wildlife Services
LFD	Lateral Flow Device
LGCA	Loliondo Game Controlled Area
NCAA	Ngorongoro Conservation Area Authority
NGOs	Non-Governmental Organizations
^{o}C	Degree Celsius
OD	optic density
OIE	World Organisation for Animal Health
PBS	Phosphate - Buffered Saline
PCP-PPR	Progressive Control Pathway for PPR
PCR	Polymerase Chain Reaction
PI	Percentage Inhibition
PPR	Peste des Petits Ruminants

PPR-GCES	Global Strategy for the Control and eradication of Peste des Petits Ruminants
PPRV	Peste des Petits Ruminants Virus
PVS	Performance of Veterinary Services
qPCR	Quantitative Polymerase Chain Reaction
RNA	Ribosomal Nucleic Acid
RP	Rinderpest
RT-PCR	Reverse Transcription – Polymerase Chain Reaction
RVC	Royal Veterinary College
SACIDS	Southern Africa Centre for Infectious Diseases Surveillance
SADC	Southern Africa Development community
SLAM	Signalling Lymphocyte Activation Molecule
SRD	Small Ruminants Disease
SUA	Sokoine University of Agriculture
TADs	Transboundary Animal Diseases
TANAPA	Tanzania National Park Authority
TAWA	Tanzania Wildlife Authority
TAWIRI	Tanzania Wildlife Research Institution
TVLA	Tanzania Veterinary Laboratory Agency
UK	United Kingdom
VACNADA	Vaccination Against Neglected Animal Diseases
WTO	World Trade Organization

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Peste des petits ruminants (PPR) is a highly contagious and economically important viral disease of domestic and wild small ruminants (FAO and OIE, 2015). It can also cause severe disease and mortality in some wild artiodactyl species and is a threat to biodiversity conservation (Hoffmann *et al.*, 2012; Munir, 2014; Pruvot *et al.*, 2020). It is a highly transmissible transboundary animal disease (TAD), with potentially devastating impacts on small ruminants, as morbidity and mortality rates can be up to 100% in naive populations (Abu Elzein *et al.*, 2004; Kumar *et al.*, 2014; Sharma *et al.*, 2015). The disease is caused by PPR virus (PPRV), a morbillivirus belonging to the family *Paramyxoviridae* which shares genetic and antigenic characteristics with rinderpest (RP) virus (Diallo *et al.*, 2007; Munir *et al.*, 2012; Kumar *et al.*, 2014; Rahman *et al.*, 2018).

The virus is classified into 4 genetically distinct lineages (I, II, III and IV) based on sequence analysis of fusion protein (F) and (N) genes (Banyard and Parida, 2015). This grouping of PPRV into lineages is of paramount importance in analysing in country and global spread of PPRV. Geographically, lineage I and II are found in West and Central Africa, whereas lineage III is in East Africa and Southern part of Middle East (Banyard *et al.*, 2010; Kivaria *et al.*, 2014) and (Kwiatek *et al.*, 2011). However, the vaccine is cross protective to all lineages. Lineage IV is the main lineage found in the Middle East and South Asia, in both wild and domestic small ruminants (Abu Elzein *et al.*, 2004; Munir, 2014). More recently, it has been found in Africa as well in Tanzania. In Tanzania, in addition to lineage IV also II and III have been found circulating into different areas of the

country. Peste des petits ruminants (PPR) virus mainly causes disease in sheep and goats, livestock species kept by small-holder farmers and pastoralists in African and Asian low and middle-income countries. These countries have approximately 94% of the global goat population and 73% of the global sheep population (FAO and OIE, 2015). Goats and sheep are an important animal resource for farmers in arid and semi-arid areas because of their low cost, and their ability to survive on sparse pastures and withstand drought (Silanikove, 2000). From natural and experimental research (Lembo *et al.,* 2013), it has been shown that cattle in close proximity to infected goats and sheep can be infected with PPRV, although they do not appear to be susceptible to clinical disease. It has been reported that cattle and domestic pigs become sub-clinically infected (Anderson and McKay, 1994; Kumar *et al.,* 2013; Sen *et al.,* 2014; Taylor and Barrett, 1946) but recent experimental work has shown that domestic pigs can be clinically infected and transmit PPRV (Schulz *et al.,* 2018). Antelopes and other wild ruminant species can be infected by PPRV, while the outcome of infection is variable depending on the species, the population and conditions in which the animals are living, whether captive or wild. A wide range of wildlife species develop antibodies with or without a clinical disease (Kock 2006; Kinne *et al.,* 2010; Lembo *et al.,* 2013; Munir, 2014; Kock *et al.,* 2015; Mahapatra *et al.,* 2015; Orynbayev *et al.,* 2016; Fernandez Aguilar *et al.,* 2018; 2020; Pruvot *et al.,* 2019).

Peste des petits ruminants virus is transmitted by contact with infectious materials from sick to healthy susceptible animals (Kihu *et al.,* 2016; Couacy-Hymann *et al.,* 2007; Fournié *et al.,* 2018). The virus can be transmitted directly via the respiratory route between animals with the possibility of pasture or water contamination as a source of transmission (Parida *et al.,* 2015). The virus concentration is high in secretions such as saliva, oral/nasal discharges, urine and faeces of the infected animals (Parida *et al.,* 2015). In domestic sheep and goats the virus is shed by sick animals prior to the development of

clinical signs and for up to 10 days after the onset of signs (Truong *et al.*, 2014). Peste des petits ruminants virus spreads to different other tissues of the body via regional lymphoid tissues, then infects the lymphocytes and infection spreads throughout the body via both the lymphatic and vascular systems (Kumar *et al.*, 2014). In sheep and goats the incubation period ranges from four to six days and the severity of disease varies depending on a number of factors including the lineage of PPRV, species, breed and the immune status of the animals (Jagtap *et al.*, 2012; Kumar *et al.*, 2014). In severe cases in naive susceptible populations of domestic goats and sheep, the clinical disease is usually acute, with pyrexia (41 to 42°C) and high proportion of animals in the flock show clinical signs at the same time (Truong *et al.*, 2014*)*. Three to five days post-infection animals become depressed, anorexic and develop serous oculo-nasal discharges, followed by mucopurulent discharges partially occluding the nostrils and matting the eyelids (Muse *et al.*, 2012; Omani *et al.*, 2019). Within four days of the onset of fever, the gums become hyperaemic and erosive lesions develop in the oral cavity with excessive salivation (Munir *et al.,* 2013). These lesions later become necrotic with a caseous grey diptheritic membrane. Watery and blood stained diarrhoea is common (Ugochukwu *et al.*, 2019) as well as pneumonia and coughing (Chauhan *et al.*, 2009).

The transmission of PPRV is often associated with trade in live sheep and goats at markets where animals from different sources are brought into close contact, mixing infected and naïve animals (Swai *et al.*, 2009; Munir, 2014; Abubakar and Munir, 2014; Baron *et al.*, 2017). Rangeland systems (including domestic and wild species), watering points, use of common markets and slaughter points provide opportunity for mixing of animals from different production systems and can become the focal for epidemic spread of PPRV (Swai *et al.*, 2011; Abubakar and Munir, 2014). The long distance movement of livestock during drought periods increases the likelihood of interaction of livestock from different

places and between livestock and wildlife at grazing and watering points (Mdetele *et al.,* 2014). These conditions facilitate the spread and maintenance of PPR. Moreover, infection of wildlife, likely via spill-over of PPRV from domestic animals, may result in serious consequences to population health of rare and threatened species, wildlife economy and ecosystems (Travis *et al.,* 2011; Munir, 2014; Kock *et al.,* 2015).

Up to the year 2000, there was no history of presence of PPR in Tanzania (Wambura, 2000; Karimuribo *et al.,*2010), The disease was first suspected in northern pastoral areas of Tanzania towards the end of 2008 (Swai *et al.,* 2009), confirmed and officially reported to the World Organization for Animal Health (OIE) on 27th January 2009. By the end of 2010 the disease had further spread to eastern and southern parts of the country (Muse *et al.,* 2012; Kivaria *et al.,* 2014). In East African countries, PPR was first reported in Ethiopia and Sudan (Taylor, 1984), confirmed in Kenya in 2007 although suspected since 1992 (Gitao *et al.,* 2014), Uganda in 2007 (Ruhweza *et al.,* 2010; Nkamwesiga *et al.,* 2019). In Burundi confirmed outbreak was diagnosed in 2017 (Niyokwishimira *et al.,* 2019). In Democratic Republic of Congo (DRC) the disease was confirmed in 2012, no clinical cases have been reported in Malawi, Mozambique and Zambia (Britton *et al.,* 2019). There are no reports of PPR outbreaks in Rwanda (Torsson *et al.,* 2016). Since the emergence of PPR in Tanzania, there have been vaccination campaigns to limit its impact on livestock keepers, yet outbreaks continue to occur. Lack of effective surveillance means to show how and where the virus is persisting increases the challenges of selecting important areas to direct the limited resources. Vaccination is usually applied in response to outbreaks based on availability of funds, with the aim to reduce livestock keepers' immediate losses due to the disease. However, low levels of vaccination coverage could be contributing to virus persistence and maintenance in the country. A more pragmatic,

research driven control programme is needed to halt PPRV persistence and spread in Tanzania, East Africa, Asia and Europe.

Of recent, at international and national levels there have been efforts of controlling PPR aiming at eradicating the disease globally by 2030. Global Strategy for the control and eradication of Peste des petits ruminants (PPR-GCES) was officially adopted on the 2nd of April 2015 in Abidjan, Côte d'Ivoire, describing the rationale for controlling and eradicating PPR, general principles and the tools to be used (PPR GCES, 2015). Regional Economic Communities as well are in the move to develop National and Regional Strategies which need to be aligned to the PPR-GCES Roadmaps and strategies. Recently Tanzania has also developed National PPR eradication plan to control and eradicate the disease in the country (National PPR Control and Eradication strategy 2017). Therefore, the aim of this study was to determine the effect of livestock movements and interactions with wildlife in maintenance and spread of PPRV in country and globally in order to complement existing efforts on establishment of effective plans for control and eradication of PPR.

1.2 Problem Statement

Peste des petis ruminants was first reported in Tanzania since 2008 and efforts to control and /or contain this economically and socially important disease have not been fruitful. Despite the control programme being imposed in Northern Zone areas of the country where the disease was reported first, the disease has continued to spread to the southern part of the country and become endemic in Tanzania. Therefore, there is a need to understand drivers for PPR spread and causes of failure to contain the disease at source despite the efforts and resources used since the first report of the disease. Moreover, there is a need to understand PPR spread in relation to small ruminants movements and

interactions with wildlife in Tanzania. The information generated from this study will be important in designing proper plans and implementations of the control and eradication programs for PPR in Tanzania and other countries where small ruminants interact and coexist with wild ruminants. The presence of PPR in Tanzania is a major obstacle to the development of the national livestock industry because of its adverse effects on livestock production, productivity and on trade of animals and animal products into lucrative export markets. Peste des petits ruminats is endemic in Tanzania; the affected area is extensive and lack of proper information of disease makes funding for control and eradication unrealistic considering the presence of uncontrolled livestock movements and large populations of wildlife in regular contacts with small ruminants. Harnessing on the growing enthusiasm for PPR control globally, regionally and in individual states, are factors favoring this study because it will complement efforts on understanding of PPR epidemiology and controlling the disease. However, the extent to which uncontrolled livestock movements, wildlife livestock interaction and other factors relate to PPR outbreaks is not known. A study is needed to establish the relationship of PPR outbreaks in different geographic areas with livestock movements and the role of wildlife in maintaining and spreading of the disease. This information is necessary during implementation of control and eradication programmes of the disease in Tanzania and other endemic countries.

1.3 Justification of the Study

Since the introduction of PPR in Tanzania in 2008 (Swai *et al.*, 2009), the disease has contributed significantly to economic and social losses to the livestock keeping communities (Mdetele *et al.*, 2015). Efforts and resources have been and continue to be used to control this disease, targeting both where it was first described in the Northern

Zone and high risky areas all over the country. In addition to that, there is a threat of disease to cross over into other countries of the Southern African Development Community (SADC) Where PPR has not been reported. The study aims at establishing the current disease situation in relation to livestock movement patterns, interaction with wildlife and drivers for occurrence of outbreaks. This information will be of paramount importance to policy makers and development partners in planning control strategies by selecting and directing properly limited resources.

It is clear that a number of wildlife species are susceptible to PPR. However the role of wildlife species in the epidemiology of PPR is uncertain (Lembo *et al.,* 2013). Information on spatial epidemiology of PPR in relation to small ruminants' movement and interactions with Wildlife in Tanzania will contribute to scientific understanding of the PPR epidemiology across ecosystems, which can have effect on decision making in relation to animal movement control and vaccination for PPR control and eradication. Moreover, it can be used to validate control programmes and ways of handling PPR outbreaks in different ecosystems.

1.4 Hypothesis and Research Questions

Hypothetically this study will determine whether PPR spread and maintenance in Tanzania is associated with small ruminant movement and interactions with wild animals. The following research questions were considered during the course of the study;-

i. How did PPR occur and subsequently spread in Tanzania?
ii. Which agro-ecological factors favor maintenance and spread of PPR in Tanzania?
iii. What are the clinical variations of PPR cases in livestock and factors favoring PPR outbreaks in wildlife livestock interface area?

iv. What are the differences in the magnitude of PPR between wildlife that are in close contact and those far away from livestock?

1.5 Objectives of the Study

1.5.1 General objective

The overall objective of the proposed research is to improve the understanding of PPR spread and maintenance in relation to livestock movements and interaction with wildlife, information necessary for proper planning of control and eradication of the disease affecting sheep and goat stock and livelihoods of small-scale farmers.

1.5.2 Specific objectives

i. Critical review on the incursion, spread and maintenance of PPR in Tanzania.

ii. Compare the magnitude of PPR prevalence among districts of different agro-ecological zones of Tanzania.

iii. To describe PPR outbreaks in livestock in the greater Serengeti ecosystem.

iv. To compare PPR seroprevalences among wildlife species in different habitats of Serengeti ecosystem.

CHAPTER TWO

Published paper I

Review of Peste des Petits Ruminants Occurrence and Spread in Tanzania

Mdetele, D.,[1*] Komba, E.,[2] Misago, S.,[3] Misinzo, G.,[4] Kock, R.[5**] and Jones, B.A.[5 **]

[1]Department of Veterinary Services, Ministry of Livestock and Fisheries P.O. Box 2870, Dodoma, Tanzania

[2]Department of Veterinary Medicine, College of Veterinary medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3021, Chuo Kikuu, Morogoro, Tanzania

[3]National Institute for Medical Research, Tanga Medical Research Centre, Tanzania

[4]Department of Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical sciences, Sokoine University of Agriculture, P.O. Box 3019, Chuo Kikuu, Morogoro, Tanzania, [5]Royal Veterinary College, Hawkshead Lane, Hatfield, Herts, AL9 7TA, UK.

animals

MDPI

Review

Review of Peste des Petits Ruminants Occurrence and Spread in Tanzania

Daniel Pius Mdetele [1,*], Erick Komba [2], Misago Dimson Seth [3], Gerald Misinzo [4], Richard Kock [5,†] and Bryony Anne Jones [5,†]

[1] Department of Veterinary Services, Ministry of Livestock and Fisheries, P.O. Box 2870, 25526 Dodoma, Tanzania
[2] Department of Veterinary Medicine and Public Health, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3021, Chuo Kikuu, 25523 Morogoro, Tanzania; komba.erick@gmail.com
[3] National Institute for Medical Research, Tanga Medical Research Centre, 25527 Tanga, Tanzania; sethmdj@gmail.com
[4] Department of Veterinary Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical sciences, Sokoine University of Agriculture, P.O. Box 3019, Chuo Kikuu, 25523 Morogoro, Tanzania; gerald.misinzo@sacids.org
[5] Royal Veterinary College, Hawkshead Lane, Hatfield, Herts AL9 7TA, UK; rkock@rvc.ac.uk (R.K.); bryony.jones@apha.gov.uk (B.A.J.)
* Correspondence: daniel.mdetele@sacids.org
† Equal contributions.

Simple Summary: Peste des petits ruminants (PPR), caused by PPR virus (PPRV), is a transboundary animal disease of sheep and goats that has a significant impact on farmer's livelihoods, food and nutritional security; and threatens susceptible wildlife. This review compiled information on the introduction and spread of PPR in Tanzania, from published and unpublished sources. PPR was first confirmed in Tanzania in 2008, but could have been present earlier, based on antibody detection in archived sera. The virus was probably introduced to northern Tanzania through cross-border movement of sheep and goats, and afterwards spread to eastern, central and southern Tanzania through movement of animals by pastoralists and traders. Genome sequencing shows that there have been several introductions of PPRV and it is now considered to be endemic. PPR has not been observed in cattle, camels or wildlife, but sera collected from these species contain PPRV antibodies, indicating virus exposure, probably through contact with infected sheep and goats. Some challenges for PPR control in Tanzania include the spread of the disease through small ruminants movements for pastoralism and trade, and limited veterinary services for disease surveillance and vaccination. The socio-economic impact of PPR justifies investment in a comprehensive disease eradication programme.

Abstract: Peste des petits ruminants (PPR) is an important transboundary animal disease of domestic small ruminants, camels, and wild artiodactyls. The disease has significant socio-economic impact on communities that depend on livestock for their livelihood and is a threat to endangered susceptible wild species. The aim of this review was to describe the introduction of PPR to Tanzania and its subsequent spread to different parts of the country. On-line databases were searched for peer-reviewed and grey literature, formal and informal reports were obtained from Tanzanian Zonal Veterinary Investigation Centres and Laboratories, and Veterinary Officers involved with PPR surveillance were contacted. PPR virus (PPRV) was confirmed in northern Tanzania in 2008, although serological data from samples collected in the region in 1998 and 2004, and evidence that the virus was already circulating in Uganda in 2003, suggests that PPRV might have been present earlier than this. It is likely that the virus which became established in Tanzania was introduced from Kenya between 2006–7 through the cross-border movement of small ruminants for trade or grazing resources, and then spread to eastern, central, and southern Tanzania from 2008 to 2010 through movement of small ruminants by pastoralists and traders. There was no evidence of PPRV sero-conversion in wildlife based on sera collected up to 2012, suggesting that they did not play a vectoring or bridging

check for updates

Citation: Mdetele, D.P.; Komba, E.; Seth, M.D.; Misinzo, G.; Kock, R.; Jones, B.A. Review of Peste des Petits Ruminants Occurrence and Spread in Tanzania. *Animals* **2021**, *11*, 1698. https://doi.org/10.3390/ani11061698

Academic Editors: Felix Njeumi, Paula Menzies and Maria Teresa Manfredi

Received: 19 March 2021
Accepted: 28 May 2021
Published: 7 June 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

role in the establishment of PPRV in Tanzania. PPRV lineages II, III and IV have been detected, indicating that there have been several virus introductions. PPRV is now considered to be endemic in sheep and goats in Tanzania, but there has been no evidence of PPR clinical disease in wildlife species in Tanzania, although serum samples collected in 2014 from several wild ruminant species were PPRV sero-positive. Similarly, no PPR disease has been observed in cattle and camels. In these atypical hosts, serological evidence indicates exposure to PPRV infection, most likely through spillover from infected sheep and goats. Some of the challenges for PPRV eradication in Tanzania include movements of small ruminants, including transboundary movements, and the capacity of veterinary services for disease surveillance and vaccination. Using wildlife and atypical domestic hosts for PPR surveillance is a useful indicator of endemism and the ongoing circulation of PPRV in livestock, especially during the implementation of vaccination to control or eliminate the disease in sheep and goats. PPR disease has a major socio-economic impact in Tanzania, which justifies the investment in a comprehensive PPRV eradication programme.

Keywords: Peste des petits ruminants; Peste des petits ruminants virus; transboundary animal diseases; epidemiology; surveillance; sheep; goat; small ruminant

1. Introduction

Peste des petits ruminants (PPR) is a highly contagious and economically important viral disease of domestic small ruminants [1]. It can also cause severe disease and mortality in some wild artiodactyl species and is a threat to biodiversity conservation [2–4], and can infect other atypical domestic species such as cattle, camels and pigs. It is caused by *Small ruminant morbillivirus* (commonly known as PPR virus) of the genus *Morbillivirus* and the family *Paramyxoviridae*, and has been classified into four genetically distinct lineages (I, II, III and IV) based on a partial sequence analysis of the fusion protein (F) and nucleoprotein (N) genes [5,6]. PPR virus (PPRV) was first identified in Côte d'Ivoire, West Africa, in 1942, and it is currently believed to be endemic across much of West, Central, North and East Africa, the Middle East and Central, South and East Asia [7]. Geographically, lineages I and II have been found predominantly in West and Central Africa, and lineage III has been found predominantly in East Africa and the Middle East [8]. Lineage IV is the main lineage found in Asia [9], both in wild and domestic small ruminants [10–12], and more recently it has been found in Africa [5,13,14].

Clinically, the disease in sheep and goats is characterised by a high fever, catarrhal ocular discharges, mucopurulent nasal discharges and erosive stomatitis in the early stages, followed by severe enteritis and bronchopneumonia [15]. The morbidity and mortality rates in PPRV endemic areas are lower compared to the rates observed in PPRV epidemics in non-endemic areas [1]. Mortality is higher in young animals compared to adults in both endemic and epidemic settings, and goats tend to be more severely affected than sheep [15,16]. Similar clinical signs have been observed in PPR outbreaks affecting various wild ruminant species in Asia, in captive, managed free-range, and wild living populations [2,4]. PPRV appears to spillover from infected domestic sheep and goats into wild animals that are in proximity, leading to infection and, in some cases, clinical disease [3,4,10,17]. The virus also causes sub-clinical infection in cattle, which develop antibodies against PPRV [18,19].

PPRV is transmitted primarily through direct contact between infected and susceptible animals, therefore communal grazing areas and live animal markets are important places for the spread of the virus [8,20]. Large amounts of infective virus are excreted in secretions and discharges from the eyes, nose and mouth, as well as in faeces [16,21,22], and the primary route of infection is respiratory, by short-range aerosols generated by sneezing and coughing [23]. The virus is fragile, so transmission of the virus by fomites is unlikely [23]. Animals that are infected with PPRV start to develop antibodies from seven to ten days post-infection [16]. Those animals that recover from infection develop lifelong immunity

that is fully protective against reinfection [23], and vaccination with live attenuated PPRV vaccine also provides lifelong protection [24,25]. The offspring of immune animals have protective maternal antibodies for up to three to four months of age [26–28].

Africa and Asia have approximately 80% of the global sheep and goat population [29]. In low and middle-income countries, goats and sheep are an important resource for smallholder farmers and pastoralists, especially in arid and semi-arid areas because of their low cost, and their ability to survive on sparse pastures and withstand drought [30].

In eastern Africa, PPR disease was first reported in Sudan in 1972 [31] and then in the horn of Africa, Ethiopia in 1994 [32]. In East Africa, Kenya officially confirmed the occurrence of PPR disease in 2007, although it is possible that disease incursions in northern districts occurred during the 1990s based on clinical and serological evidence [33,34]. This was a time of active rinderpest circulation [35] and PPRV serology may have been unreliable with cross-reactivity between these closely related viruses in the absence of attempts for differentiation by cross-neutralisation [34]. Uganda confirmed PPR disease officially for the first time in 2007 [36,37], but evidence for infection in Uganda was first reported in small ruminants in 2003 and in wildlife based on serology in 2004 [38]; while Tanzania first confirmed PPR disease the following year, in 2008 [39]. The first confirmation of PPR disease in the Democratic Republic of the Congo (DRC) was in 2012, and in Burundi in 2017 [40]. However, there have so far been no confirmed cases of PPR disease in Rwanda [41], but serological evidence suggests PPRV could be present although it is still unconfirmed by virus detection [42]. No clinical disease has been reported in Malawi, Mozambique or Zambia, which border Tanzania to the south [43].

The Global Strategy for the Control and Eradication of PPR (PPR GCES) was officially adopted in 2015 by the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO). The strategy describes the rationale for controlling and eradicating PPRV, the general principles, and the tools to be used [1]. A strategy for Africa has been developed by the African Union Inter-African Bureau for Animal Resources (AU-IBAR), and regional strategies that are aligned with the global strategy have been developed by the Inter-governmental Authority on Development (IGAD) for the Greater Horn region, and the Southern African Development Community (SADC) [43–45]. At the national level, Tanzania is in the process of developing a national PPRV control and eradication plan.

The aim of this review is to describe the first confirmed introduction of PPRV to Tanzania and its subsequent persistence and spread to different parts of the country. This review will contribute to an improved understanding of the history of PPRV in Tanzania and will provide insights into how the virus is maintained and spread, that will contribute to more effective planning and implementation of the PPRV control and eradication programme by the Tanzania Veterinary Services and the veterinary services of neighbouring countries.

2. Materials and Methods

A narrative review method was used, and a literature search for peer-reviewed published papers and grey literature was carried out for all documentation up to 2020 using the online publication databases; CAB Direct (https://www.cabdirect.org/ accessed on 2 March 2020), PubMed (https://www.ncbi.nlm.nih.gov/pubmed/ accessed on 20 March 2020) and Google Scholar (https://scholar.google.com/ accessed on 20 March 2020). The search strategy focused on reports of PPR disease surveillance, investigations in Tanzania, and the location of PPR disease events, prior to and after the first confirmation of PPRV in 2008, using the search terms "peste des petits ruminants" and "Tanzania". For the published articles, only articles written in English were considered and 19 publicly available papers were included in this review. For the unpublished reports, documents in English and Swahili languages were considered, and 10 reports were included in this review. The titles of papers and documents generated by the searches were reviewed and the full texts of those that were relevant were obtained for full text review. If the title did

not provide enough detail for acceptance or rejection, the abstract was also scrutinised. To be considered for review, the papers had to provide information on the occurrence of PPRV infection and/or disease in Tanzania. The first author, along with two other co-authors selected the articles to be reviewed, and the remaining authors were involved in providing additional literature.

In addition to publicly available literature, the annual reports (2008–2018) of the seven Zonal Veterinary Investigation Centres (VIC) were reviewed for information about PPRV, and formal and informal reports, letters and emails containing information about PPR surveillance or disease events between 2007 and 2018 were obtained from the files of the three Zonal VICs and zonal veterinary laboratories that are responsible for disease surveillance in North and Central Tanzania (Northern, Lake and Central Zones) (Figure 1). Veterinary officers at the Northern, Lake and Central Zonal VICs and Laboratories, who had been involved in PPR surveillance and investigations, were contacted by phone, or were met in person to obtain information about current or unpublished studies.

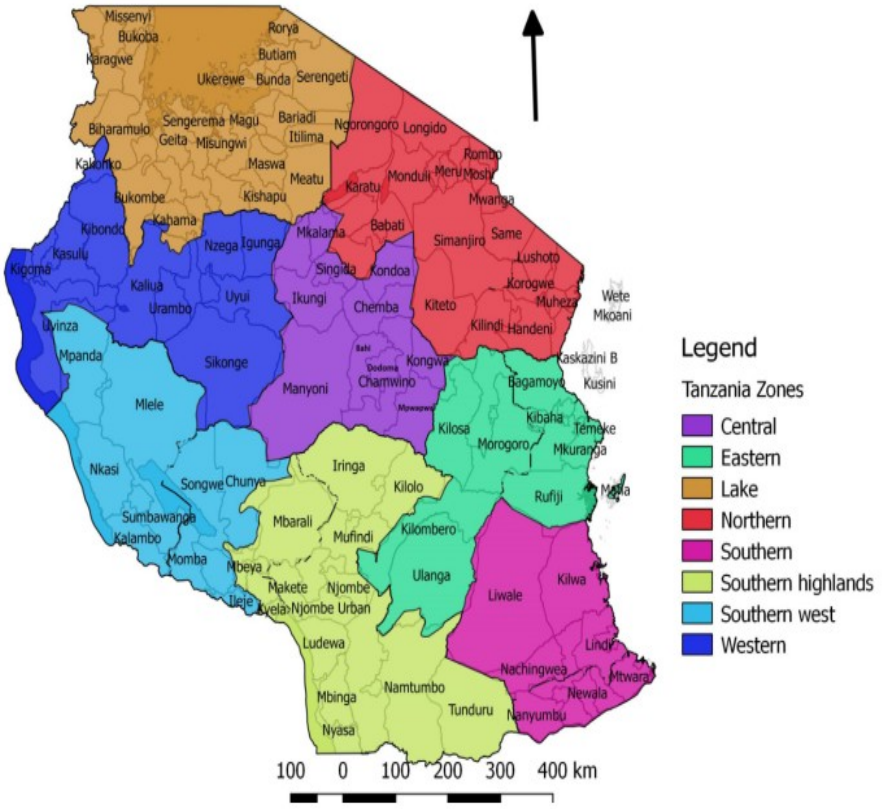


Figure 1. Map of Tanzania showing the seven surveillance zones and their respective districts. Each zone has a Veterinary Investigation Centre and a laboratory.

3. Results and Discussion

The results of this review are presented as follows. First, we describe the evidence available for the possible presence of PPRV prior to official confirmation in 2008. Then we describe the PPR disease outbreak that occurred in 2008 in northern Tanzania, followed by the emergence of PPRV disease in southern Tanzania between 2009 and 2010, in eastern Tanzania in 2010, and in Central Tanzania in 2014. This is followed by descriptions of the evidence of PPRV infection in other domestic animals and in wild species, the molecular characterization of PPRV obtained from cases in different parts of the country, PPRV serological surveys, and evidence of PPRV co-infection with other pathogens in Tanzania.

Finally, we describe the socio-economic impact and the main challenges for PPRV control in Tanzania.

3.1. Evidence of PPRV in Tanzania Prior to Confirmation of Infection in 2008

Wambura (2000) reported that, prior to 1998, no PPR outbreaks had been reported, and no serological surveys had been carried out in Tanzania [46]. In 1998, a national cross-sectional serological survey for PPRV and rinderpest virus was conducted. A total of 3134 serum samples were collected from sheep and goats that were randomly selected from all 20 regions in all seven surveillance zones of mainland Tanzania (Figure 1). The samples were analysed for both rinderpest virus and PPRV antibodies using the rinderpest virus haemagglutinin (H) competitive enzyme-linked immunosorbent assay (c-ELISA) and the PPRV H c-ELISA [47], and all were negative in both tests, including 520 samples from Arusha region where the first confirmed outbreak of PPRV was subsequently detected in 2008 [46]. Rinderpest virus is a morbillivirus that is closely related to PPRV, for which cattle were the main host, but sheep and goats could be infected with rinderpest virus and develop antibodies. The global eradication of rinderpest was achieved in 2011. PPRV antibodies could cross-react with rinderpest virus c-ELISA, therefore it was necessary to run both assays in parallel to distinguish between PPRV and rinderpest virus antibodies.

Serological surveillance was conducted in wildlife populations across East and Central Africa between 1994 and 2004 during the rinderpest eradication programmes of the Pan-African Rinderpest Campaign (PARC) and the Pan-African Programme for the Control of Epizootics (PACE) [48]. While the main objective was surveillance for rinderpest virus antibodies, 967 serum samples from 20 wild artiodactyl species were also tested for PPRV antibodies on plates coated with a PPRV recombinant nucleocapsid protein (N) c-ELISA at the Agricultural Research Centre for International Development (CIRAD), France. A subset was also tested by PPRV H c-ELISA at The Pirbright Institute, UK. A rinderpest virus and PPRV cross-neutralisation test was carried out for PPRV N c-ELISA positive samples, and 31 were considered to be PPRV sero-positive based on higher titres in the PPR virus neutralization test (VNT) compared to rinderpest VNT. These positive samples were collected from African buffalo (*Syncerus caffer*) and eland (*Taurotragus oryx*) in southern Ethiopia and Kenya. Out of the 967 samples, 127 were collected in Tanzania from buffalo (92), eland (15), giraffe (*Giraffa camelopardalis*) (9), hartebeest (*Alcelaphus buselaphus*) (4), kudu (*Tragelaphus strepsiceros*) (2), oryx (*Oryx gazella*) (1), roan antelope (*Hippotragus equinus*) (2), sable antelope (*Hippotragus niger*) (1) and topi (*Damaliscus lunatus jimela*) (1) in the Serengeti and Mkomazi protected areas in northern Tanzania, and in the Ruaha and Katavi protected areas in southern Tanzania. All of these samples were PPRV sero-negative by N c-ELISA and one buffalo and two eland from Mkomazi and one buffalo from Serengeti were PPRV sero-positive by H cELISA but also positive for rinderpest by H c-ELISA and serum neutralization, so these results were considered to be due to cross-reaction [48].

Karimuribo et al. (2010) carried out a retrospective investigation to determine whether PPRV might have been present in northern Tanzania prior to the official confirmation in 2008 [49]. A total of 198 serum samples were retrieved from the serum bank of the VIC in Arusha and were analysed for PPRV antibodies by N c-ELISA as described by Swai et al. (2009) [50]. These serum samples had been collected from goats and sheep during investigations in the Ngorongoro district of suspected Rift Valley fever (RVF) cases in 1998 (52 serum samples) and suspected PPR cases in 2004 (21 samples), and during a toxoplasmosis survey in 2004 covering the Ngorongoro, Monduli, Mbulu and Karatu districts (125 samples). All the samples collected in 1998 were sero-negative, but 17.1% of sera collected in 2004 were sero-positive (25 of 146 samples). There was a higher sero-prevalence (71.4% of 21 samples) for the serum samples that were collected from suspected PPR cases compared to those collected for investigations of other diseases (5.7%, χ^2 test p value < 0.001). Moreover, the animals sampled in the Ngorongoro district had a higher sero-prevalence (18.3% of 104 samples) compared to the animals sampled from other areas (6.4% of 94 samples). Interviews were conducted with key informants from

the Ngorongoro district veterinary office, VIC Arusha and the epidemiology unit in the Ministry of Livestock Development and Fisheries, who provided information on reports and investigations of PPR-like diseases in Ngorongoro district in 1995, 2002, 2004 (when the above serum samples were collected), 2006 and 2008 (when PPRV was confirmed). The authors concluded that PPRV had been present in northern Tanzania at least four years prior to the official confirmation in 2008 [49]. An alternative explanation was cross-reactivity to rinderpest virus in small ruminants. Rinderpest disease in cattle was confirmed in Karatu district, and in Ngorongoro Conservation Area and Loliondo in Ngorongoro district in 1997, and sero-surveillance showed a low sero-prevalence of rinderpest antibody in small ruminants at that time [51]. Spillover of rinderpest virus to small ruminants could account for cross-reactive PPRV sero-positivity and for the PPR-like clinical signs observed, when rinderpest virus was present and actively circulating in cattle in northern Tanzania and across the border in Kenya. If the animals that were sero-positive in 2004 were alive at the time of rinderpest circulation, it is possible that the positive results were a cross-reaction due to the presence of rinderpest virus antibody.

3.2. The First Confirmed PPRV Disease Cases in Northern Tanzania, 2008

Various sources describe reports of a PPR-like disease occurring in Ngorongoro district in Arusha Region of northern Tanzania in late 2007 and early 2008 [39,49,52]. In March 2008, high mortality was reported among sheep and goats in Ngorongoro district, leading to an investigation by VIC Arusha during which a total of 112 sheep and goats were clinically examined, some post mortem examinations were carried out, and serum samples were collected and found to be antibody negative to PPRV [39]. Unfortunately, serological analysis at the Central Veterinary Laboratory (CVL) was delayed for approximately six months due to a lack of diagnostic test kits [52]. Reports of sheep and goat mortality continued in Ngorongoro and Mara districts, so in June 2008 a further investigation was carried out. A total of 404 serum samples were collected in Ngorongoro district, of which 129 (31.9%) were sero-positive, and 84 sera were collected in Mara district, of which all were sero-negative [39].

The serological results suggested the presence of PPRV in northern Tanzania, therefore a cross-sectional serological survey was carried out between August 2008 and July 2009 in 12 purposively selected districts of northern Tanzania that were perceived to be at risk of PPRV due to their proximity to southern Kenya, where PPR disease had recently been reported in Kajiado district. The aim of the survey was to investigate whether there was evidence of PPRV circulation and the spatial extent of the spread [20,50]. A total of 3478 serum samples were collected from healthy unvaccinated goats (2182) and sheep (1296) from pastoralist or agropastoralist flocks in 79 randomly selected villages from four districts in Arusha region (Ngorongoro, Karatu, Longido, Monduli), one district in Kilimanjaro region (Siha), two districts in Manyara region (Mbulu, Simanjiro), and five districts of Tanga region (Korogwe, Lushoto, Mkinga, Muheza and Tanga). During the survey, cases of suspected PPR disease were observed in Ngorongoro, Monduli, Longido and Siha districts, with signs of diarrhoea, fever, and nasal discharge, and a post mortem examination of two goats found signs of bronchopneumonia, swollen mesenteric lymph nodes and hyperaemic small intestines [39]. Using the H c-ELISA [47], the overall sero-prevalence was 22.1% (95% Confidence Interval: 20.7–23.5%). The district level sero-prevalence varied from 0 to 88%, with a higher sero-prevalence in pastoral compared to agro-pastoral districts (Figure 2). In Arusha region, Longido district had the highest seroprevalence (88%) followed by Ngorongoro (55%), Monduli (35%) and Karatu districts (29%). In Manyara region, Mbulu district had a sero-prevalence of 47%, but Simanjiro was sero-negative. Siha district in Kilimanjaro region had a sero-prevalence of 43%. The district-level seroprevalence in Tanga Region was lower, ranging from 0 to 11% [39]. The high PPRV seroprevalence in an unvaccinated population suggested natural transmission of PPRV in these northern districts [50].

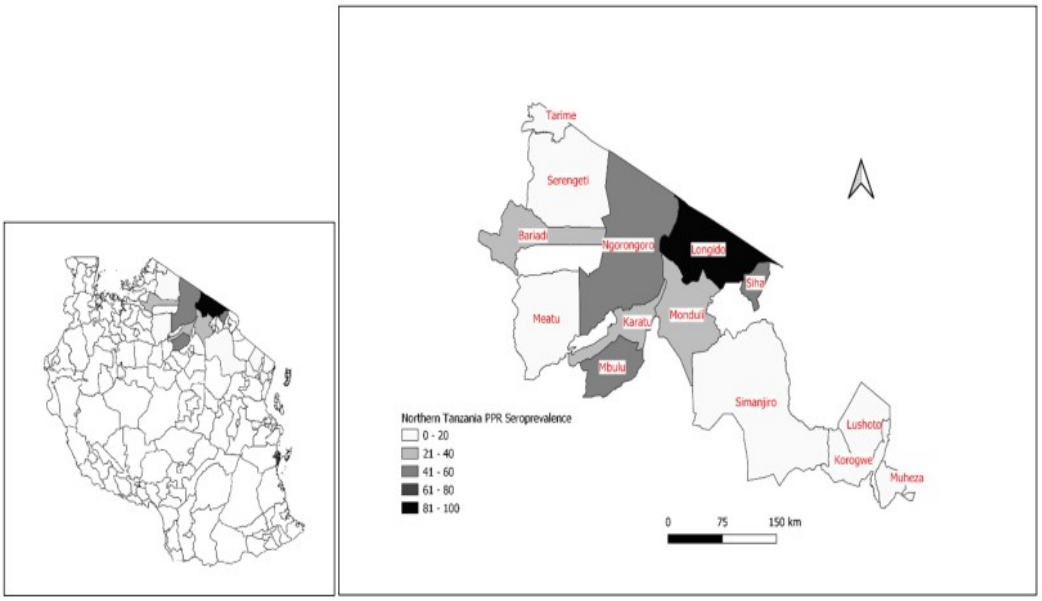


Figure 2. Results of cross-sectional PPRV serological surveys of districts in the Northern and Lake Zones of Tanzania carried out in 2008–2009: map of Tanzania with surveyed districts shaded in grey (**left**); map of surveyed districts indicating district-level seroprevalence (**right**). Source of data: [20,39].

In December 2008, tissue samples including spleen, liver, mediastinal lymph nodes, and whole blood were collected from an outbreak in Soitsambu, Ngorongoro district, and sent to the World Reference Laboratory for PPR at CIRAD, France. PPRV was confirmed by reverse transcription polymerase chain reaction (RT-PCR), using primers targeting the nucleoprotein (N) gene, showing that this virus belonged to lineage III [39,52]. The first occurrence of PPRV in Tanzania was officially reported to the OIE on 27th January 2009 (https://wahis.oie.int/ accessed on 14 January 2020). The source of the virus was suspected to be through the cross-border movement of animals from Kenya, where PPRV had been confirmed in the border districts of Narok and Kajiado in 2008 [50,52,53].

During the same time period, VIC Mwanza received reports of suspected PPR outbreaks in the Lake Zone and alerted the District Veterinary Officers (DVOs) of all the districts in the zone, in particular those bordering the Northern Zone (Meatu, Bariadi, Serengeti, Bunda and Magu), bordering Kenya (Tarime and Rorya), and bordering Uganda (Misenyi and Karagwe). In December 2008, purposive serological surveys were conducted in Serengeti and Tarime districts of Mara region, and Bariadi and Meatu districts of Shinyanga region. In each of these districts, 10 villages were selected, and in each village, five livestock keepers were visited, and blood samples collected from 10 goats and sheep. A total of 1945 serum samples were collected and analysed by c-ELISA (type of cELISA not specified), out of which 189 (9.7%) were sero-positive. The proportion of animals that were sero-positive was low in animals sampled in Serengeti (0.8%), Tarime (3.1%) and Meatu districts (1.2%), but 33.7% of the animals sampled in Bariadi district were sero-positive, which suggests that PPRV had been circulating in this district, since no PPRV vaccination had been carried out in this area [20]. Bariadi district lies to the west of Ngorongoro district in the Northern Zone.

In response to the confirmed outbreaks of PPR in 2008, the Veterinary Services of Tanzania collaborated with the non-governmental organization (NGO), VETAID to carry out a vaccination campaign in February 2009 in Ngorongoro, Karatu, Mbulu, Monduli, Arumeru and Arusha districts of Arusha Region (62,000 doses). In the following year (January to April 2010), 3.2 million doses of vaccine were delivered to goats and sheep in the remaining districts of northern Tanzania by the Tanzania Veterinary Services with the assistance of FAO [52]. Another vaccination campaign was carried out from mid-2010 to

2012 by the Tanzania Veterinary Services with the support of the Vaccination for Control of Neglected Animal Diseases in Africa (VACNADA) project (a European Commission funded project that was coordinated by the African Union Interafrican Bureau for Animal Resources). Vaccinations were carried out in the regions neighbouring Kenya, covering districts in the Northern Zone (Kiteto, Simanjiro, Longido, Meru, Arusha DC, Monduli, Ngorongoro and Siha) and the Lake Zone (Tarime, Rorya, Bunda and Serengeti) [54] (Figure 2).

Since 2009, there have been several published reports of confirmed PPR disease in the Ngorongoro district [17,55–57]. In addition, unpublished CVL reports obtained from VIC Arusha provided information on confirmed PPR disease in Ngorongoro in 2017, and in the Kiteto district, Manyara region in 2015.

In August 2011, Mwanza VIC received a report of an outbreak of disease in sheep and goats causing mortality in villages around Lake Kitangiri in Meatu and Kishapu districts of Shinyanga region (Qwari, B., 2011, "Goat and sheep mortalities investigation at Meatu and Kishapu district, unpublished report submitted to the Officer In-Charge, VIC Mwanza, dated 9 August 2011). The clinical signs that were described by livestock keepers and observed by investigators were swelling of the mandibular area and dewlap, conjunctivitis, lacrimation, nasal discharge, coughing, sneezing, lesions on the lips and nostrils, diarrhoea, and emaciation. The mortality rate was approximately 30%. The farmers reported that sheep were more severely affected than goats, and that animals grazing around Lake Kitangiri were more affected. The disease was new to the farmers, and they associated the outbreak with the presence of scavenging domestic pigs wallowing in the lake. One post mortem examination was carried out on an affected goat, which showed an emaciated and dehydrated carcass with congested lungs, hyperaemic trachea, and bronchi containing froth. Based on the clinical history, clinical signs and post mortem signs, and the cessation of cases after vaccination was carried out, it was concluded that PPRV could be the cause, but serum and tissue samples submitted to the CVL were all negative.

In summary, during 2008, PPR disease was confirmed in Ngorongoro district of northern Tanzania, and serological surveys in the Northern and Lake Zones demonstrated that PPRV had been circulating widely in northern Tanzania. It is likely that PPRV was introduced to northern Tanzania from infected areas of southern Kenya through the movement of live infected animals via trade or cross-border movements of flocks, and the delays in the diagnosis of PPRV and the implementation of control measures led to virus spread across the north of the country [52]. Vaccination campaigns during 2009–2012 reduced the number of outbreaks, but PPRV outbreaks continued to occur on an annual basis due to limited vaccination coverage.

3.3. Emergence of PPRV Disease in Southern Tanzania, 2009–2010

PPR disease in sheep and goats was first suspected in southern Tanzania in December 2009, based on interviews conducted with local animal health workers and veterinarians [39]. The first village to be affected was believed to be Likuna village in Newala district, Mtwara region, which borders with Mozambique (Figure 1). The source of infection was suspected to be from 70 goats that were purchased by a livestock trader from the Pugu livestock market, near Dar es Salaam. Three days after arrival, some of these goats died, so to limit losses, the trader sold some of the animals to nearby villages where suspected PPR cases subsequently occurred [39,58]. By March 2010, cases were occurring in the nearby Tandahimba and Masasi districts, probably due to the movement of small ruminants through the local markets, and there was high morbidity and mortality [39,58]. Serological analysis using PPRV H c-ELISA showed that 84% of 180 serum samples collected in Tandahimba district were positive, while only 3% of 125 samples collected in Masasi district were positive [39]. During the same period, another outbreak was also suspected in Namtumbo district in Ruvuma region of southern Tanzania, which neighbours Malawi and Mozambique.

In October 2010, risk-based sero-surveillance was carried out in districts in southern Tanzania bordering Mozambique, Malawi, and Zambia. Out of the 720 sera collected, 36.9% were PPRV antibody positive [39]. Although positive serology in the absence of PPRV vaccination suggested that PPRV was circulating, PPRV was not confirmed as the cause of the disease in southern Tanzania until Muse et al. (2011) investigated clinical cases of suspected PPR in two villages in Tandahimba district in March 2011 [59]. PPRV ribonucleic acid (RNA) was detected in ocular and nasal swabs and tissues from post mortem examination by RT-PCR. Livestock keepers had observed increasing mortality in goats, while sheep were less severely affected. The clinical signs were mainly respiratory with only a few animals affected showing diarrhoea. The main signs observed were fever, lacrimation, nasal discharge, respiratory distress and coughing, and oral and nasal ulceration. Mortality was higher in kids than adults, but all age groups were affected. Nodular skin lesions were observed all over the body, which suggests that the animals were co-infected with sheep and goat pox virus [58].

During March to May 2011, Muse et al. (2012) carried out a serological survey in Newala and Tandahimba districts [58]. In eight purposively selected villages with suspected cases, interviews were conducted with owners of affected goats and sheep, and five randomly selected goats and sheep were blood sampled from each household. Out of 79 households visited, 81.3% in Tandahimba district reported that they had seen suspected PPR cases in their flocks compared to 19.4% in Newala district. A total of 216 serum samples were collected and tested for the PPRV antibody by c-ELISA (type of cELISA not specified). The overall seroprevalence was 31% (95% CI 24.9–37.6%), but a higher proportion of samples collected in Tandahimba were positive (55.5%) than in Newala district (5.7%). Given that no PPRV vaccination had been carried out in southern Tanzania, the high seroprevalence suggested PPRV transmission in the area.

In order to determine whether PPRV had been circulating in southern Tanzania prior to 2009, Mbyuzi et al. (2014) conducted PPRV H c-ELISA on 477 serum samples collected from goats and sheep in 2007 from Lindi and Mtwara regions for RVF surveillance, and 504 sera collected from the affected flocks during the 2009 outbreak investigations in Mtwara region [60]. All the sera collected in 2007 were PPRV antibody negative, providing no evidence of PPRV circulation at that time, while 28.8% of goats and 35.7% of sheep were positive in the 2009 sera samples.

In summary, suspected PPR disease occurred in 2009 in southern Tanzania and was confirmed to be caused by PPRV in 2011. It is likely that the virus was introduced by animal trade from another part of Tanzania via a live animal market in Dar es Salaam and was subsequently spread through small ruminant trade and communal grazing. A delay in the confirmation of the diagnosis led to delays in the implementation of control measures which contributed to the persistence and spread of PPRV [39].

3.4. PPRV Disease in Eastern Tanzania, 2010

In April 2010, there was high mortality of sheep and goats from an unknown disease in Ulanga district in the southern part of Morogoro region in the Eastern Zone of Tanzania [39]. Investigations indicated that this could have been caused by PPRV, which could have been introduced to the area by the northward movement of migratory pastoralists from Mtwara and Lindi regions. Out of 200 serum samples collected, 76% were positive by PPRV H c-ELISA [39]. In June–July 2010, outbreaks of suspected PPR disease were reported in the Mvomero district in the north of Morogoro region [61]. An investigation was conducted and clinical cases in goats and sheep were found, with signs of nasal discharge, diarrhoea and oral ulcers, while enlarged and congested gastrointestinal and bronchial lymph nodes were observed during post mortem examinations. PPRV was confirmed in tissue samples by RT-PCR. In order to control the outbreaks in the Southern and Eastern Zones, the Directorate of Veterinary Services supported ring vaccination through the provision of PPRV vaccine to Mtwara and Ruvuma regions (252,000 doses), and to Ulanga in the Morogoro region (74,000 doses), which appeared to limit the spread of the disease [52].

In addition, between November 2014 and January 2015 an outbreak investigation was conducted in Morogoro district where mortality and abortions had been reported in goats. Samples were collected from six goats with clinical signs from Morogoro Urban and Mvomero districts [62]. These samples were subjected to molecular analysis for PPRV identification and PPRV was identified by RT-PCR in one goat from each site. The outbreak started a few days after one farm introduced some apparently healthy goats that had been purchased in Mkongeni market in Mvomero district, and neighbouring farms that were in contact in the grazing area were also affected.

3.5. PPRV Disease in Central Tanzania, 2014

In the Dodoma region of Central Tanzania, field and laboratory reports held at Dodoma Veterinary Investigation Centre provided evidence of PPRV circulation in this area from 2014. In August 2014, a disease outbreak was reported among small ruminants in Kongwa district, Dodoma Region, in which 19.5% of the affected animals died (Theodata, 2014 Field work report at Central Zone Investigation Centre, unpublished report). The animals were observed to have diarrhoea, profuse nasal discharge, dyspnoea and anorexia. Out of 30 serum samples collected, 53.3% were positive for PPRV by PPRV c-ELISA (the type of c-ELISA was not specified). In the same month, 65 serum samples were collected from Singida Rural District Council, in the Singida region to the west of Dodoma, of which 20% were sero-positive. All the animals that were blood-sampled originated from areas where the last vaccination had been carried out in 2012. In May 2016, there was a disease outbreak in small ruminants in the Bahi district, Dodoma Region, in which animals showed signs of lacrimation, nasal discharge and diarrhoea. Tissue samples, whole blood and nasal swabs were collected and submitted to the Sokoine University of Agriculture for analysis. PPRV RNA was detected in the blood samples, based on information obtained from laboratory results filed at the Central Zone Investigation Centre (ZVC/DOM/Lab/samp/2016/4/19/2—ZVC Dodoma laboratory result file). In addition, 50 serum samples from the same area were submitted to the Tanzania Veterinary Laboratory Agency (TVLA) and 94% were positive for PPRV by c-ELISA (TVLA/CIDB/PPR/001/2016—ZVC Dodoma laboratory result file).

3.6. Evidence of PPRV Infection in Camels and Cattle

Serological evidence of PPRV infection in camels was found in a study carried out in northern Tanzania [63]. This study was carried out in eight districts of Arumeru, Longido, Monduli, Mwanga, Same, Hai, Simanjiro and Kilindi in four regions of northern Tanzania. During June and August 2010, a total of 193 serum samples were collected from 14 camel herds and tested for PPRV antibody by H c-ELISA. The overall seroprevalence was 2.6% (5/193) providing evidence of PPRV infection in camels, although no clinical disease had been observed.

In 2011, when investigating the role of cattle in PPRV epidemiology in northern Tanzania, 266 serum samples were collected from cattle living in close proximity to sheep and goats in randomly selected pastoralist households in eight villages in the northern part of Ngorongoro district, where PPR disease had been confirmed in sheep and goats in 2008 [19]. The overall seroprevalence was 17.3% by H c-ELISA and N c-ELISA, and seroprevalence was higher among cattle aged over three years that had been alive during the 2008 outbreak (26.7%) compared to cattle aged less than two years that had been born after 2008 (5.9%). The seroprevalence at the village level ranged from 7% to 48% [19]. The authors concluded that it was likely that there had been cross-species transmission of PPRV from small ruminants to cattle. In addition, Kivaria et al. (2013) report that PPRV antibody was detected in cattle sharing grazing with small ruminants in the Mkuranga district, Pwani region in eastern Tanzania [39]. It should be noted that in both the camel and cattle serological studies, possible cross-reaction of the PPRV c-ELISA with other morbilliviruses that these species could have been exposed to was not explored, such as canine distemper virus.

3.7. Evidence of PPRV Infection in Wildlife

Lembo et al. (2013) also carried out PPRV serological analysis of archived serum samples collected from wild species in northern Tanzania, to investigate possible spillover of PPRV from sheep and goats to wildlife [19]. A total of 243 African buffalo, 59 Thomson's gazelle (*Eudorcas thomsonii*) and 6 Grant's gazelle (*Nanger granti*) were sampled from the Serengeti National Park (NP) to the west of Ngorongoro district, Ngorongoro Conservation Area (NCA), in the southern part of Ngorongoro district, and from Arusha and Tarangire NPs to the east of Ngorongoro. Samples were also collected from 23 buffalo in Katavi NP, western Tanzania [19]. The samples were collected during wildlife immobilization operations conducted for rinderpest surveillance, research activities and conservation management prior to 2008 and during 2008–2012. The samples were analysed by PPRV H c-ELISA (Biological Diagnostic Supplies Limited [BDSL]) and none were sero-positive, providing no evidence of PPRV infection in these wild ruminant species in these ecosystems during this time period [19].

In June 2014, a study was carried out looking for evidence of a spillover of PPRV from domestic to wild ruminants in NCA [17]. Eleven sampling sites were purposively selected where resident wild ruminants were present in close proximity to domestic small ruminants, sharing grazing and water sources. Clinical cases of PPR disease were confirmed in sheep and goats in the area during the study period by a PPRV rapid diagnostic test (BDSL) and RT-PCR. Serum samples were collected from 46 wild animals. Overall, 63% of the animals sampled were positive by PPRV H c-ELISA (BDSL), and all herds and species had at least one sero-positive animal, except for Thomson's gazelle for which only one animal was sampled: African buffalo (10 sampled from 2 herds, 50% positive); Grant's gazelle (30 sampled from 8 herds, 67% positive); Thomson's gazelle (1 sampled, 0% positive); wildebeest (*Connochaetes taurinus*) (2 sampled from one herd, 50% positive); impala (*Aepyceros melampus*) (3 sampled from one herd, 100% positive). These results provided the first evidence of PPRV infection in wildlife in Tanzania, although no PPR clinical cases have been reported so far in wild species in East Africa except some unverified reports in free ranging wild species from Sudan [64]. The apparently high seroprevalence in wildlife in close proximity to sheep and goats compared to sero-negative wild animals within national parks [19] supports the hypothesis of a spillover of infection from domestic to wild animals [17]. The possible cross-reaction of the PPRV c-ELISA with other morbilliviruses that these wild species could have been exposed to was not explored, such as canine distemper virus.

3.8. Molecular Biology of PPRV in Tanzania

As described earlier, tissue samples collected from goats with clinical signs of PPR from the Ngorongoro district during 2008 were PPRV RT-PCR positive [39]. A partial nucleoprotein gene sequence was obtained, and phylogenetic analysis showed that it was a lineage III virus, and that it clustered with PPRV detected in East Africa (Ethiopia in 1994, Sudan in 1972) as well as from the United Arab Emirates (1986) and Oman (1983).

In 2012–2013, Kgotlele et al. (2014) confirmed PPRV by RT-PCR in samples collected from goats with suspected PPR clinical signs in three villages of Ngorongoro district (Meshili, Piyaya and Malambo) and two villages of Mvomero district (Kauzeni and Dakawa) in Morogoro region in eastern Tanzania [55]. Phylogenetic analysis of partial nucleoprotein gene sequences indicated that the PPRV obtained from northern and eastern Tanzania clustered together in lineage III and were most closely related to PPRV from Ethiopia (1996). However, the sequence obtained in 2009 from Ngorongoro was not included in the analysis, so the relationship to this earlier PPRV in Tanzania is not shown.

During the outbreak investigations conducted by Namtimba in 2014–2015 in Morogoro district in Morogoro region, phylogenetic analysis of the nucleoprotein gene nucleotide sequences showed that the PPRV involved in the outbreak clustered with lineage III viruses and was closely related to those reported by Kgotlele et al., (2014) in Dakawa and Ngorongoro (Figure 3) [61,62].

Misinzo et al. (2015) carried out partial nucleoprotein gene sequencing on the PPRV positive samples collected by Muse et al. (2012) from the 2011 PPRV outbreak in the Tandahimba district, southern Tanzania [14]. Phylogenetic analysis showed that one sequence clustered in lineage II with the Nigeria 75/1 vaccine strain, and a second sequence was most closely related to a lineage IV virus from Turkey, and clustered with viruses from the Middle East, and from Central, East and North Africa.

During the June 2014 study of PPRV at the livestock–wildlife interface in Ngorongoro district, Mahapatra et al. (2015) obtained PPRV RT-PCR positive samples from PPR cases in domestic sheep. Based on partial nucleoprotein gene sequencing it was determined that this was a lineage II virus that clustered with the lineage II virus obtained from southern Tanzania in 2011 [17].

During the investigations of PPR outbreaks in Ngorongoro district in June 2015, partial nucleoprotein gene sequences were obtained from PPRV real-time RT-PCR (RT-qPCR) positive samples [56]. Phylogenetic analysis showed that the sequences obtained clustered with the lineage III PPRV obtained by Kgotlele et al. (2014) from Ngorongoro in 2013, and these shared a common ancestry with a cluster of viruses from Kenya (2011), Uganda (2012 and 2018), and DRC (2018), and a cluster of viruses from Tanzania, including from Morogoro [55].

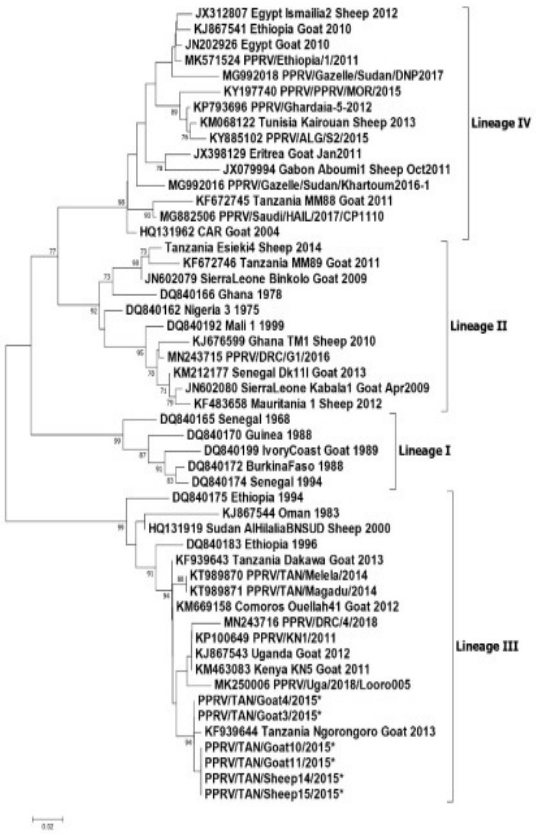


Figure 3. Phylogenetic tree reproduced from Jones et al. (2020) [56]. Neighbour-joining tree constructed on the basis of partial nucleoprotein gene sequences of the peste des petits ruminants virus (PPRV). The tree shows the relationships among African PPRV isolates. The scale bar indicates the nucleotide substitutions per site. The Kimura 2-parameter model with the percentage of replicate trees in which the associated taxa clustered together in the 1000 bootstrap replicates is shown next to the branches. The taxon name of the sequences retrieved from the GenBank contains the accession number followed by the name of the country and the year of isolation. The Tanzanian sequences appearing in this diagram and described in this paper are.

- Lineage II: Tanzania Esieki4 Sheep 2014 [17], KF 672,746 Tanzania, MM89 Goat 2011 [14].
- Lineage III: KF939644 Tanzania Ngorongoro goat 2013 [55], KT989870 PPRV/TAN/Melela/2014 and KT989871 PPRV/TAN/Magadu/2014 [62], KF939643 Tanzania Dakawa Goat 2013 [55]. The six sequences marked by an asterisk (PPRV/TAN/Goat 3, 4, 10, 11 and Sheep 14, 15/2015 are GenBank accession numbers: MT181842-47 obtained from Ngorongoro 2015 [56].
- Lineage IV: KF 672,745 Tanzania, MM88 Goat 2011 [14].

In summary, lineage III PPRVs that share a common ancestry with lineage III viruses from Kenya, Uganda and DRC have been identified in northern and eastern Tanzania between 2008 and 2015 (Figure 4). Lineage IV virus has been detected once from southern Tanzania, and closely related lineage II viruses have been detected in the north and the south. This diversity of viruses indicates that there have been multiple introductions of PPRV into Tanzania. PPRV lineage III viruses predominate in East Africa (Sudan, Ethiopia, Kenya, Uganda, Burundi and DRC), while lineage IV viruses have been detected in East (Uganda, South Sudan, Sudan and Ethiopia), Central and North Africa [65]. The detection of lineage II viruses could be due to the circulation of a virus that is a variant of the Nigeria 75/1 vaccine strain, or it is possible that the results are due to laboratory contamination [65]. It is therefore important to continue to obtain samples for genome sequencing from PPRV outbreaks in all parts of Tanzania to better represent the PPR viruses that are circulating, and to gain insights into how PPRV is spread within the country and across borders.

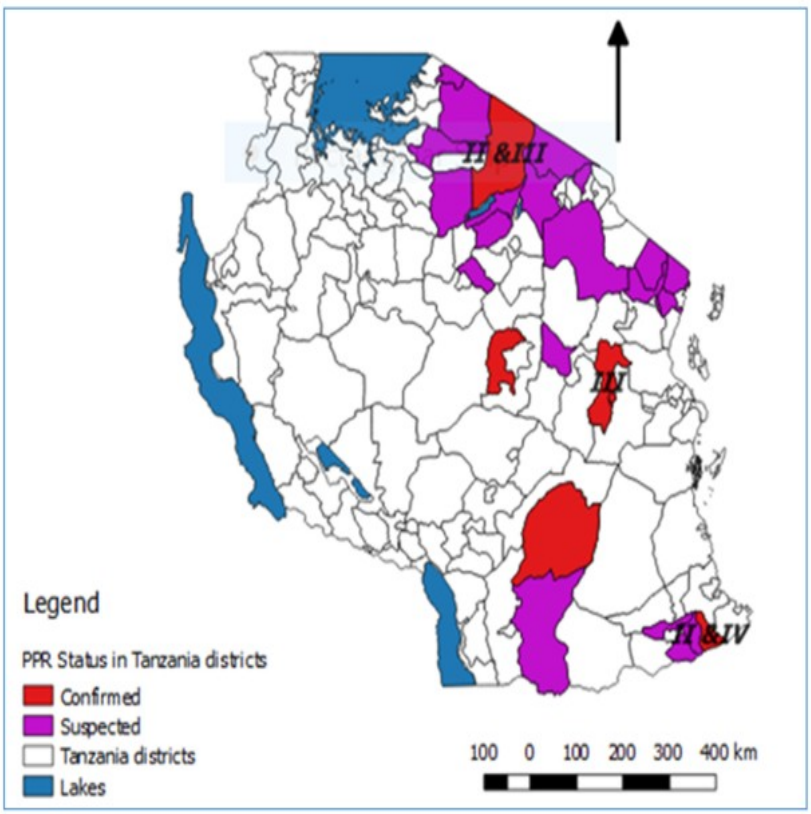


Figure 4. Map of Tanzania showing the districts where PPRV disease was laboratory confirmed or suspected during the period 2008 to 2018. The places where PPRV partial genome sequences were obtained are indicated by the relevant lineage numbers.

3.9. PPRV Serological Surveys

In 2013, a PPRV serological survey was carried out with the aim of determining the sero-prevalence of PPRV in different ecological zones [66]. A total of 32 districts with large sheep and goat populations that had not previously been surveyed, and where no PPRV vaccination had yet been carried out, were purposively selected, of which three (9.4%) were from the coastal zone, 12 (37.5%) were from the semi-arid zone and 17 (53.1%) were from the plateau zone. The serum samples used for analysis were retrieved from the zonal veterinary laboratory. The retrieved samples and documents did not indicate the specific age and vaccination status of the sampled animals. A total of 2490 serum samples were analysed by H c-ELISA, and the overall sero-prevalence was 20.1%, while the district-level sero-prevalence ranged from 0% to 75%. The PPRV sero-prevalence in districts in the semi-arid and coastal zones was found to be significantly higher compared to those in the plateau ecological zone [66]. The possibility that some of the sampled animals could have been brought in from infected and/or vaccinated areas and therefore were exposed to PPRV prior to arrival cannot be excluded.

Kgotlele et al. (2016) also described a PPRV serological survey that was carried out in 2013 that aimed to determine the sero-prevalence in 11 of the 25 mainland regions of Tanzania, and therefore the distribution of PPRV in Tanzania [61]. Samples were collected from healthy goats and sheep in 103 villages in 35 districts (no further details of the survey design were provided). In 2015, three additional regions were surveyed: Arusha, Manyara and Kilimanjaro, in which 15 villages from 3 districts were sampled. Out of 3838 sera collected (2886 goats, 952 sheep), 26.0% were sero-positive by N c-ELISA and there was no difference in sero-prevalence between the sheep and goats. At the regional level, sero-prevalence varied from 2.6% to 70.0%, with the highest prevalence in Northern, Central and Eastern zones, which could be partly attributed to the vaccination that was carried out in these areas (the vaccination status of the sampled animals was not mentioned). All the regions sampled in the Lake Zone and Southern highlands had low sero-prevalence (<10.0%), while the two regions in the Western Zone, which was previously believed to be free of PPRV, had seroprevalences of 9.0% (Kigoma) and 11.4% (Tabora).

In summary, the serological surveys concluded that the occurrence of PPRV in the country shows ecological zone pre-disposition, with sero-prevalence being higher in semi-arid and coastal zones characterised by low relative humidity. They confirmed the presence of antibodies against PPRV in sheep and goats in regions of Tanzania that previously had little to no data on the disease, which indicated that PPRV had spread within Tanzania with the possibility of spread across the border to neighbouring countries.

3.10. PPRV Co-infections with Other Pathogens

Several studies of PPR outbreaks have described co-infections of PPRV and other pathogens. During 2015, Jones et al. (2020) investigated a series of reports of a PPR-like disease in pastoralist small ruminant flocks in Ngorongoro district [56]. Out of 33 outbreak investigations, ten flocks were found to be PPRV positive by either a PPRV rapid diagnostic test (PPRV-RDT, BDSL Irvine Ltd., Irvine, UK) and/or RT-qPCR, and two of these flocks were also positive for bluetongue virus (BTV) by RT-qPCR. Based on clinical signs (but not laboratory confirmation), cases of goat pox and contagious caprine pleuropneumonia (CCPP) were also observed in the area during the study. The confirmed PPRV outbreaks showed a diversity of clinical signs—some flocks primarily showed a respiratory syndrome, while others had a diarrhoea syndrome or a more classical PPR syndrome with nasal discharge, coughing and diarrhoea. In line with the clinical syndrome that they observed, the livestock keepers used different local names for the confirmed cases—local terms for respiratory disease, diarrhoea disease or rinderpest-like disease [56].

Muse et al. (2012) described nodules all over the body as a clinical sign of PPR during the 2011 southern Tanzania PPR outbreak, but a photograph of a clinical case shows the classical cutaneous nodules of goat pox infection [58]. Similarly, Kgotlele et al. (2014) described cutaneous nodules as a clinical sign of PPR disease in Ngorongoro and

Morogoro, and provided a photograph of typical cutaneous nodules of goat pox [61]. All of these outbreaks were confirmed as PPRV by RT-PCR; therefore, it is likely that there was co-infection with Capripox virus. In 2016, an investigation was carried out to determine the aetiology of a respiratory disease outbreak causing high mortality in sheep and goats in Loliondo Division of Ngorongoro district [57], part of the study area covered by Jones et al. (2020). Four of the affected villages were visited, where the most frequent clinical signs were nasal discharge and diarrhoea, in some cases tinged with blood, as well as skin nodules and loss of body condition. Nasal swabs collected from 61 animals were examined by a multiplex RT–qPCR method to detect PPRV, *Mycoplasma capricolum subspecies capripneumoniae* (Mccp, causative agent of CCPP), *Pasteurella multocida*, and Capripoxvirus (causative agent of sheep and goat pox). Out of the 61 samples, 38 (62%) were positive for one or more of the four pathogens, and eight of these (21%) had co-infections. The most frequently detected pathogen was *P. multocida* (33% of animals), while 15% had Capripoxvirus, 13% had PPRV and 13% had Mccp. Out of these, one animal (2%) was co-infected with PPRV and Mccp, one was co-infected with PPRV and Capripox, and one was co-infected with PPRV and *P. multocida*. Four animals (7%) were co-infected with Mccp and Capripox [57]. These studies demonstrate the difficulty that field veterinarians face in making a clinical diagnosis of small ruminant respiratory and diarrhoea syndromes, and the importance of laboratory diagnosis to support differential diagnosis and the identification of mixed infections, which is necessary for determining appropriate interventions, especially in relation to the PPRV eradication programme.

3.11. Socio-economic Impact of PPR Disease

Two studies of the socio-economic impact of PPR disease have been carried in pastoralist and agro-pastoralist production systems of Tanzania. The first was conducted in 2012 in two agropastoralist districts—Tandahimba district in Mtwara Region and Ulanga district in Morogoro Region (Msuya and Kimera, 2013: Peste des petites ruminants (PPR): assessment of socio-economic impacts in the Republic of Tanzania. Working document, No 1., 2013 unpublished report), while the second study was carried out in 2017 in the Ngorongoro district in the Northern Zone which is a pastoralist production system, and the Kibaha and Bagamoyo districts in the Eastern Zone, which have agropastoralist production systems [67]. Based on a survey of households in PPR disease-affected villages, the first study estimated that the average loss per household due to PPR disease was 735,820 Tanzanian shillings (490.6 US$). Extrapolating these losses to the national level, it was estimated that the cumulative losses due to PPR disease between 2009 and 2012 in the eight regions that had been affected was approximately 101.8 billion Tanzanian shillings (US$ 67.9 million), due to small ruminant mortality, milk loss, abortion, premature culling and the cost of disease control. In the second study, data on the losses due to PPR disease were collected using a household survey, which included losses due to mortality, milk loss, abortion and weight loss and cost of control. A spreadsheet model was developed which estimated that the average annual loss per household due to PPR disease in goats was 1,920,924 Tanzanian shillings (approximately 850 US$) and due to PPR disease in sheep, the loss per household was 1,162,562 Tanzanian shillings (approximately 530 US$). These studies suggest that PPR is an economically important disease in Tanzania causing a high economic burden and threatening food security, especially among pastoral communities, because it causes the loss of small ruminants as productive assets, reduces herd size, and causes a loss of potential income. Figure 5 shows the population of sheep and goats per region of Tanzania. There is considerable variation in the numbers of small ruminants by region, and therefore in the potential socio-economic impact of the PPR disease. No benefit-cost analysis of PPRV eradication in Tanzania has been carried out, but based on a global benefit-cost analysis [68], it is likely that the eradication of PPRV from Tanzania would be economically beneficial compared to allowing the disease to continue to be endemic, due to the high losses caused by mortality and morbidity and the cost of on-going control measures. Reduced losses

due to the PPR disease would support wealth creation and poverty alleviation, justifying investment in regional and global PPRV eradication.

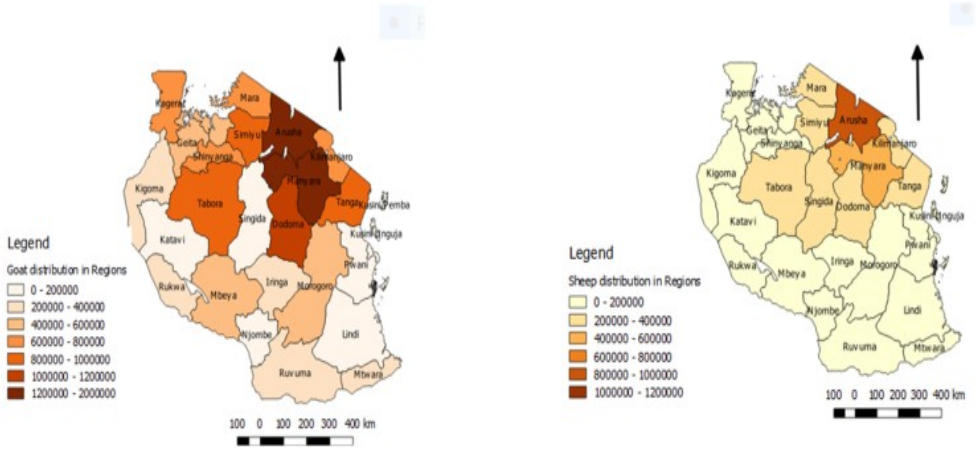


Figure 5. Estimated goat (**left**) and sheep (**right**) populations in different regions of Tanzania. Source of data: Ministry of Livestock and Fisheries, 2020.

3.12. Challenges for the Control and Eradication of PPRV in Tanzania

This review of the literature related to the introduction and spread of PPRV in Tanzania has highlighted some key challenges that will need to be taken into account when planning interventions for the control and eradication of PPRV in Tanzania. Many of these challenges are also relevant for other emerging and endemic infectious diseases of livestock in the country.

3.12.1. Timely Diagnosis and Intervention

For transboundary animal disease control, early detection and rapid response is fundamental to the control of a newly introduced pathogen. In the case of the introduction of PPRV to Tanzania, this review has shown that there were considerable delays between the receiving of the initial reports of high mortality disease to conducting outbreak investigations and obtaining laboratory results to confirm the diagnosis, and finally, the implementation of the disease control interventions. As a result, in each area of Tanzania where PPRV emerged, infection spread widely before any control measures were carried out, and it is likely that PPRV is now endemic in many parts of Tanzania.

3.12.2. Diagnosis of PPR Disease

PPR disease shows a range of clinical signs that are similar to the signs of other diseases, such as contagious caprine pleuropneumonia, pasteurellosis, bluetongue, and foot-and-mouth disease. It was therefore not surprising that the livestock keepers and veterinarians had difficulty in differentiating the newly introduced PPR disease from other small ruminant diseases that were already present in their areas. The confusion with other diseases, as well as co-infection of PPRV with other pathogens, made clinical diagnosis very difficult. Considering the fact that most disease diagnoses and reports are made by field officers using clinical signs, there is a need for capacity building of field personnel on PPRV clinical diagnosis and differential diagnosis, and this should be supported by the deployment of PPRV rapid test kits and sample collection equipment for laboratory diagnosis and differential diagnosis. In addition, the regional and national laboratories require the skills, equipment, and resources to carry out PPRV diagnosis and differential diagnosis to provide timely results for decision-making.

3.12.3. Extensive Production System with Mobility of Small Ruminants

Most small ruminant production in Tanzania is under extensive management, either small-holder, pastoral or agro-pastoral production, which involves the movement of animals over shorter or longer distances on a daily or seasonal basis to access pasture, water and markets, and to avoid disease [69]. These movements lead to frequent contact between flocks, within and between districts, regions and countries, which facilitates the transmission of infectious pathogens such as PPRV and leads to the spread and maintenance of disease in Tanzania [53,63]. Periods of drought can cause long distance movements of livestock to access water and grazing, and can increase the interaction between livestock and wildlife at grazing and watering points [53,70].

In the past few decades, pastoralist and agro-pastoralist production systems in Tanzania have been facing a shortage of natural pastures and water for livestock, which has been attributed to climate change and an increase in the human and livestock populations [71]. This has forced agro-pastoral and pastoral communities to migrate into different regions of Tanzania to search for pasture and water, which may have facilitated the spread of transboundary animal diseases to different parts of the country. An example of this has been the migration of pastoralists and agro-pastoralists from the Northern and Lake Zones to more humid areas in eastern and southern parts of Tanzania [69] and an increase in the livestock populations in southern Tanzania, an area that is not a traditional livestock production area [71]. A major influx of pastoralists and agro-pastoralists into the Kilombero and Usangu valleys was linked to a decline in water flow to the Great Ruaha River, which caused an energy crisis because the river is a major source of hydro-electric power generation. This forced the Government to evict the pastoralists and agro-pastoralists from the Usangu and Kilombero valleys in 2007 [72,73]. The evicted pastoralists were ordered to settle in the Lindi region, but because of a quarantine imposed in 2008 due to an RVF outbreak, some of them decided to settle in the Ruvuma and Coastal regions [71]. The movement of evicted pastoralists could have contributed to the southward spread of PPRV to the Mtwara and Ruvuma regions.

3.12.4. Transboundary Small Ruminant Movements

Tanzania has extensive land borders with eight countries in East, Central and Southern Africa, and there is unofficial movement of small ruminants across these borders for the purposes of accessing grazing or water sources, trade, or social reasons. The initial introduction of PPRV into Ngorongoro district has been attributed to the movement of pastoralist animals or the live animal trade with Kenya [39,50,52]. The detection of lineage III PPR viruses that share a common ancestry with the viruses detected in Kenya and Uganda supports this hypothesis, although the small number of PPRV sequences from East Africa is a major limitation for phylogenetic analysis. In addition to lineage III PPRV, the detection of lineage II PPRV in the north and south of Tanzania and lineage IV in the south demonstrates that there have been multiple introductions of PPRV to Tanzania. Lineage II is predominantly found in West and Central Africa and was identified in Uganda in 2007, while lineage IV is the predominant lineage in Asia and has been found in North, East and Central Africa [17].

3.12.5. Small Ruminant Trade

The movement of small ruminants for the live animal trade is a common practice in Tanzania. Livestock keepers or traders bring animals on foot from the villages to primary markets, where animals are either slaughtered, bought by livestock keepers for breeding or fattening, or bought by traders for onward transportation to secondary and tertiary markets, which may involve unofficial movement across international borders. The trade network provides opportunities for disease transmission between flocks in different geographical locations. The live animal trade was mentioned as a possible source of introduction and spread of PPRV in Tanzania [50]. In particular, goats that were bought in a market near Dar es Salaam and transported to southern Tanzania were implicated as the cause of the

PPR disease outbreak in the southern region [39,58]. Therefore, it is important for PPRV control to understand the small ruminant trade network patterns, and how the network links small ruminant populations in different parts of Tanzania.

3.12.6. Wildlife

So far, there has been no confirmation of clinical PPR disease or virus maintenance in wildlife species in Tanzania but there is clear evidence of regular spillover from infected livestock. Evidence of clinical disease and spread of PPRV amongst free-ranging wildlife species in Sudan (Dorcas gazelle—*Gazella dorcas*—which is in the same genus as Thomson's and Grant's gazelle) [64] and Asia (including impala and Thomson's gazelle) and in captivity, suggest that wildlife in the Tanzanian ecosystems are for some reason resistant to disease. This may reflect optimal environmental, ecological, and nutritional conditions for wildlife population and the overall robust health of these animals. Whilst this situation persists there is unlikely to be a risk of vectoring or bridging virus between wildlife and livestock, but if the situation changes through environmental or climatic perturbation this status may change. This has been seen with other morbillivirus infections in wildlife such as the canine distemper virus and lions in the Serengeti, where infection occurred sporadically year on year but the disease was only expressed under certain environmental conditions [74]. The continued monitoring of wildlife for disease and evidence of infection will be important during the period of control or elimination of virus under the global eradication campaign for PPRV.

3.12.7. Strengthening of Veterinary Services

The OIE Performance of Veterinary Services (PVS) reports of 2009 and 2017 highlighted that the department of veterinary services in Tanzania is faced with a number of challenges that reduce its efficiency, from financial resources to the organizational structure [75,76]. This limits the capacity for the early detection, diagnosis, and rapid response to contain a transboundary disease such as PPR. The failure of the Veterinary Services to meet the OIE, FAO and World Trade Organization standards in terms of early reporting, identification (diagnosis), surveillance activities, livestock movement, border control, and timely management of disease outbreaks could have contributed to the initial spread of PPRV and the ongoing spread and maintenance of PPRV in different areas of Tanzania. Therefore, there is a need for a further strengthening of the veterinary services chain of command, capacity building of laboratories for efficient and timely disease diagnosis, and a strengthening of cross-border surveillance activities.

4. Conclusions

In Tanzania, PPRV was first confirmed and reported to the OIE in December 2008. Serological studies and reports indicate that PPRV may have entered the north of the country periodically before 2008 but this may be confounded by the presence of rinderpest virus up until 1999 or later in the border areas with Kenya and serum cross-reactivity for both viruses. It is likely that PPRV was introduced from neighbouring countries and then spread from the north to the south in Tanzania via the live animal trade and pastoralist movements. Although PPRV antibodies have been detected in sheep and goats in all zones, the highest prevalence of disease is in the Northern Zone where there is a large population of sheep and goats. PPRV antibodies have been detected in cattle, camels, and wildlife, and although no clinical cases have been reported in any of these species, there is an ongoing risk of spillover from sheep and goats at the wildlife–livestock interface and the risk of wildlife disease outbreaks is not negligible. The review shows that PPRV is now endemic in Tanzania, which is causing persistent economic losses in the livestock sector, disturbing livelihoods, and posing a potential threat to biodiversity conservation and the wildlife economy. This justifies investment in a rapid progression to the elimination of the virus from Tanzania in coordination with other countries in the region.

Author Contributions: Conceptualization, D.P.M. and B.A.J.; methodology, D.P.M. and B.A.J.; writing—original draft preparation, D.P.M. and B.A.J.; writing—review and editing, D.P.M., E.K., M.D.S., G.M., R.K. and B.A.J.; visualization, D.P.M.; supervision, E.K., M.D.S., G.M., R.K. and B.A.J.; funding acquisition, R.K. All authors read and endorsed the final version of the manuscript.

Funding: This review was funded by the Global Challenges Research Fund of the UK government through the Biotechnology and Biological Sciences Research Council-funded project: Pathway to Peste des Petits Ruminants Virus Elimination—methods for complex ecosystems BB/P023002/1 and the Engineering and Physical Sciences Research Council (EPSRC) Development of multispecies validated serology protocols for complex ecosystems, focused on East Africa, in support of Global PPR eradication EP/T015381/1.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Unpublished reports that are cited in this review that are not publicly available, are available on request from the corresponding author.

Acknowledgments: The assistance of veterinary authorities and personnel in the zonal veterinary centres and laboratories in providing access to documents is gratefully acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAO; OIE. Global strategy for the control and eradication of PPR. In Proceedings of the International Conference for the Control and Eradication of Peste des Petits Ruminants (PPR), Abidjan, Ivory Coast, 31 March–2 April 2015.
2. Hoffmann, B.; Wiesner, H.; Maltzan, J.; Mustefa, R.; Eschbaumer, M.; Arif, F.A.; Beer, M. Fatalities in Wild Goats in Kurdistan Associated with Peste Des Petits Ruminants Virus. *Transbound. Emerg. Dis.* **2011**, *59*, 173–176. [CrossRef] [PubMed]
3. Munir, M. Role of Wild Small Ruminants in the Epidemiology of Peste des Petits Ruminants. *Transbound. Emerg. Dis.* **2013**, *61*, 411–424. [CrossRef] [PubMed]
4. Pruvot, M.; Fine, A.E.; Hollinger, C.; Strindberg, S.; Damdinjav, B.; Buuveibaatar, B.; Chimeddorj, B.; Bayandonoi, G.; Khishgee, B.; Sandag, B.; et al. Outbreak of peste des petits ruminants virus among critically endangered Mongolian saiga and other wild ungulates, Mongolia, 2016–2017. *Emerg. Infect. Dis.* **2020**, *26*, 51. [CrossRef]
5. Muniraju, M.; Munir, M.; Parthiban, A.R.; Banyard, A.C.; Bao, J.; Wang, Z.; Ayebazibwe, C.; Ayelet, G.; El Harrak, M.; Mahapatra, M.; et al. Molecular Evolution of Peste des Petits Ruminants Virus. *Emerg. Infect. Dis.* **2014**, *20*, 2023–2033. [CrossRef] [PubMed]
6. Libeau, G.; Diallo, A.; Parida, S. Evolutionary genetics underlying the spread of peste des petits ruminants virus. *Anim. Front.* **2014**, *4*, 14–20. [CrossRef]
7. Clarke, B.D.; Islam, M.R.; Abu Yusuf, M.; Mahapatra, M.; Parida, S. Molecular detection, isolation and characterization of Peste-des-petits ruminants virus from goat milk from outbreaks in Bangladesh and its implication for eradication strategy. *Transbound. Emerg. Dis.* **2018**, *65*, 1597–1604. [CrossRef]
8. Banyard, A.C.; Parida, S.; Batten, C.; Oura, C.; Kwiatek, O.; Libeau, G. Global distribution of peste des petits ruminants virus and prospects for improved diagnosis and control. *J. Gen. Virol.* **2010**, *91*, 2885–2897. [CrossRef] [PubMed]
9. Liu, F.; Li, J.; Li, L.; Liu, Y.; Wu, X.; Wang, Z. Peste des petits ruminants in China since its first outbreak in 2007: A 10-year review. *Transbound. Emerg. Dis.* **2018**, *65*, 638–648. [CrossRef]
10. Abu Elzein, E.M.E.; Housawi, F.M.T.; Bashareek, Y.; Gameel, A.A.; Al-Afaleq, A.I.; Anderson, E. Severe PPR Infection in gazelles kept under semi-free range conditions. *J. Vet. Med. Ser. B Infect. Dis. Vet. Public Health* **2004**, *51*, 68–71. [CrossRef] [PubMed]
11. Abubakar, M.; Munir, M. Peste des Petits Ruminants Virus: An Emerging Threat to Goat Farming in Pakistan. *Transbound. Emerg. Dis.* **2014**, *61* (Suppl. 1), 7–10. [CrossRef]
12. Rahman, M.Z.; Haider, N.; Gurley, E.S.; Ahmed, S.; Osmani, M.G.; Hossain, M.B.; Islam, A.; Khan, S.A.; Hossain, M.E.; Epstein, J.H.; et al. Epidemiology and genetic characterization of Peste des petits ruminants virus in Bangladesh. *Vet. Med. Sci.* **2018**, *4*, 161–171. [CrossRef] [PubMed]
13. Kwiatek, O.; Ali, Y.H.; Saeed, I.; Khalafalla, A.I.; Mohamed, O.I.; Abu Obeida, A.; Abdelrahman, M.B.; Osman, H.M.; Taha, K.M.; Abbas, Z.; et al. Asian Lineage of Peste des Petits Ruminants Virus, Africa. *Emerg. Infect. Dis.* **2011**, *17*, 1223–1231. [CrossRef] [PubMed]
14. Misinzo, G.; Kgotlele, T.; Muse, E.A.; Van Doorsselaere, J.; Berg, M.; Munir, M. Peste des Petits Ruminants Virus Lineage II and IV From Goats in Southern Tanzania During an Outbreak in 2011. *Br. J. Virol.* **2015**, *2*, 1–4.
15. Baron, M.D.; Parida, S.; Oura, C.A.L. Peste des petits ruminants: A suitable candidate for eradication? *Vet. Rec.* **2011**, *169*, 16–21. [CrossRef]
16. Truong, T.; Boshra, H.; Embury-Hyatt, C.; Nfon, C.; Gerdts, V.; Tikoo, S.; Babiuk, L.A.; Kara, P.D.; Chetty, T.; Mather, A.; et al. Peste des Petits Ruminants Virus Tissue Tropism and Pathogenesis in Sheep and Goats following Experimental Infection. *PLoS ONE* **2014**, *9*, e87145. [CrossRef] [PubMed]

17. Mahapatra, M.; Sayalel, K.; Muniraju, M.; Eblate, E.; Fyumagwa, R.; Shilinde, S.; MaulidMdaki, M.; Keyyu, J.; Parida, S.; Kock, R. Spillover of Peste des Petits Ruminants Virus from Domestic to Wild Ruminants in the Serengeti Ecosystem, Tanzania. *Emerg. Infect. Dis.* **2015**, *21*, 2230–2234. [CrossRef]
18. Abubakar, M.; Mahapatra, M.; Muniraju, M.; Arshed, M.J.; Khan, E.H.; Banyard, A.C.; Ali, Q.; Parida, S. Serological Detection of Antibodies to Peste des Petits Ruminants Virus in Large Ruminants. *Transbound. Emerg. Dis.* **2015**, *64*, 513–519. [CrossRef] [PubMed]
19. Lembo, T.; Oura, C.; Parida, S.; Hoare, R.; Frost, L.; Fyumagwa, R.; Kivaria, F.; Chubwa, C.; Kock, R.; Cleaveland, S.; et al. Peste des Petits Ruminants Infection among Cattle and Wildlife in Northern Tanzania. *Emerg. Infect. Dis.* **2013**, *19*, 2037–2040. [CrossRef] [PubMed]
20. Madege. *Peste des Petits Ruminants (PPR) and Contagious Pleuropneumonia (CBPP) Surveillance in Lake Zone, Presentation to the VACNADA Inception Workshop, Morogoro, 2011*; Archived document at Zonal Veterinary Investigation Centre: Mwanza, Tanzania, 2011.
21. Baron, J.; Bin-Tarif, A.; Herbert, R.; Frost, L.; Taylor, G.; Baron, M.D. Early changes in cytokine expression in peste des petits ruminants disease. *Vet. Res.* **2014**, *45*, 22. [CrossRef] [PubMed]
22. Munir, M.; Zohari, S.; Berg, M.; Munir, M.; Zohari, S.; Berg, M. Replication and Virulence Determinants of Peste des Petits Ruminants Virus. In *Molecular Biology and Pathogenesis of Peste des Petits Ruminants Virus*; Springer: Berlin, Germany, 2013; pp. 23–32. [CrossRef]
23. Parida, S.; Muniraju, M.; Mahapatra, M.; Muthuchelvan, D.; Buczkowski, H.; Banyard, A. Peste des petits ruminants. *Vet. Microbiol.* **2015**, *181*, 90–106. [CrossRef]
24. Singh, R.K.; Rajak, K.K.; Muthuchelvan, D.; Banyard, A.C.; Parida, S. Chapter 11 Vaccines against Peste des Petits ruminants virus. In *Peste des Petits Ruminants Virus*; Munir, M., Ed.; Springer: Berlin/Heidelberg, Germany, 2015; pp. 51–67.
25. Singh, R.P.; Bandyopadhyay, S.K. Peste des petits ruminants vaccine and vaccination in India: Sharing experience with disease endemic countries. *Virus Dis.* **2015**, *26*, 215–224. [CrossRef]
26. Awa, D.N.; Ngagnou, A.; Tefiang, E.; Yaya, D.; Njoya, A. Post vaccination and colostral peste des petits ruminants antibody dynamics in research flocks of Kirdi goats and Foulbe sheep of north Cameroon. *Prev. Vet. Med.* **2002**, *55*, 265–271. [CrossRef]
27. Balamurugan, V.; Sen, A.; Venkatesan, G.; Rajak, K.K.; Bhanuprakash, V.; Singh, R.K. Study on passive immunity: Time of vaccination in kids born to goats vaccinated against Peste des petits ruminants. *Virol. Sin.* **2012**, *27*, 228–233. [CrossRef]
28. Enchery, F.; Hamers, C.; Kwiatek, O.; Gaillardet, D.; Montange, C.; Brunel, H.; Goutebroze, S.; Philippe-Reversat, C.; Libeau, G.; Hudelet, P.; et al. Development of a PPRV challenge model in goats and its use to assess the efficacy of a PPR vaccine. *Vaccine* **2019**, *37*, 1667–1673. [CrossRef] [PubMed]
29. FAO; OIE. *Peste des Petits Ruminants Global Eradication Programme*; FAO: Rome, Italy, 2016.
30. Silanikove, N. Goat production under harsh environmental conditions: The physiological basis and the challenge. In *The Opportunities and Challenges for Enhancing Goat Production in East Africa*; Langston University: Langston, OK, USA, 2000.
31. El Hag Ali, B.; Taylor, W.P. Isolation of peste des petits ruminants virus from the Sudan. *Res. Vet. Sci.* **1984**, *36*, 1–4. [CrossRef]
32. Roeder, P.L.; Abraham, G.; Kenfe, G.; Barrett, T. Peste des petits ruminants in Ethiopian goats. *Trop. Anim. Health Prod.* **1994**, *26*, 69–73. [CrossRef] [PubMed]
33. Gitao, C.G.; Ithinji, D.G.; Gitari, R.; Ireri, G.R. The confirmation of Peste des petit ruminants (PPR) in Kenya and perception of the disease in West Pokot. *Res. Opin. Anim. Vet. Sci.* **2014**, *4*, 312–317.
34. Wamwayi, H.M.; Rossiter, P.B.; Kariuki, D.P.; Wafula, J.S.; Barrett, T.; Anderson, J. Peste des petits ruminants antibodies in east Africa. *Vet. Rec.* **1995**, *136*, 199–200. [CrossRef] [PubMed]
35. Kock, R.; Wambua, J.M.; Mwanzia, J.; Wamwayi, H.; Ndungu, E.K.; Barrett, T.; Kock, N.D.; Rossiter, P.B. Rinderpest epidemic in wild ruminants in Kenya 1993-97. *Vet. Rec.* **1999**, *145*, 275–283. [CrossRef] [PubMed]
36. Nkamwesiga, J.; Coffin-Schmitt, J.; Ochwo, S.; Mwiine, F.N.; Palopoli, A.; Ndekezi, C.; Isingoma, E.; Nantima, N.; Nsamba, P.; Adiba, R.; et al. Identification of Peste des Petits Ruminants Transmission Hotspots in the Karamoja Subregion of Uganda for Targeting of Eradication Interventions. *Front. Vet. Sci.* **2019**, *6*, 221. [CrossRef] [PubMed]
37. Ruhweza, S.; Ayebazibwe, C.; Mwiine, F.; Muhanguzi, D.; Olaho, W. Seroprevalence of Peste des Petits Ruminants (PPR) virus antibodies in goats and sheep in north-eastern Uganda. *Bull. Anim. Health Prod. Afr.* **2010**, *58*. [CrossRef]
38. Aguilar, X.F.; Mahapatra, M.; Begovoeva, M.; Kalema-Zikusoka, G.; Driciru, M.; Ayebazibwe, C.; Adwok, D.S.; Kock, M.; Lukusa, J.-P.K.; Muro, J.; et al. Peste des Petits Ruminants at the Wildlife–Livestock Interface in the Northern Albertine Rift and Nile Basin, East Africa. *Viruses* **2020**, *12*, 293. [CrossRef]
39. Kivaria, F.M.; Kwiatek, O.; Kapaga, A.M.; Swai, E.S.; Libeau, G.; Moshy, W.; Mbyuzi, A.O.; Gladson, J. The incursion, persistence and spread of peste des petits ruminants in Tanzania: Epidemiological patterns and predictions. *Onderstepoort J. Vet. Res.* **2013**, *80*, 10. [CrossRef] [PubMed]
40. Niyokwishimira, A.; Baziki, J.D.D.; Dundon, W.G.; Nwankpa, N.; Njoroge, C.; Boussini, H.; Wamwayi, H.; Jaw, B.; Cattoli, G.; Nkundwanayo, C.; et al. Detection and molecular characterization of Peste des Petits Ruminants virus from outbreaks in Burundi. *Transbound. Emerg. Dis.* **2019**, *66*, 2067–2073. [CrossRef] [PubMed]
41. Torsson, E.; Kgotlele, T.; Berg, M.; Mtui-Malamsha, N.; Swai, E.S.; Wensman, J.J.; Misinzo, G. History and current status of peste des petits ruminants virus in Tanzania. *Infect. Ecol. Epidemiol.* **2016**, *6*, 32701. [CrossRef] [PubMed]

42. Shyaka, A.; Ugirabe, M.A.; Wensman, J.J. Serological Evidence of Exposure to Peste des Petits Ruminants in Small Ruminants in Rwanda. *Front. Vet. Sci.* **2021**, *8*, 186. [CrossRef] [PubMed]
43. Britton, A.; Caron, A.; Bedane, B. Progress to Control and Eradication of Peste des Petits Ruminants in the Southern African Development Community Region. *Front. Vet. Sci.* **2019**, *6*, 6. [CrossRef]
44. African Union. *The Pan African Strategy for Control and Eradication of Peste des Petits Ruminants*; African Union—Interafrican Bureau for Animal Resources (AU-IBAR): Nairobi, Kenya, September 2015; pp. 1–3.
45. ICPALD. The IGAD Regional Peste des Petits Ruminants (PPR) Control and Eradication Strategy Presented at the Global Conference In Abijan (31 March–2 April 2015). 2016. Available online: http://icpald.org/wp-content/uploads/2016/01/Progressive-Control-and-Eradication-Strategy-1.pdf (accessed on 23 April 2019).
46. Wambura, P.N. Serological evidence of the absence of peste des petits ruminants in Tanzania. *Vet. Rec.* **2000**, *146*, 473–474. [CrossRef] [PubMed]
47. Anderson, J.; McKay, J.A. The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implications to rinderpest control programmes. *Epidemiol. Infect.* **1994**, *112*, 225–231. [CrossRef] [PubMed]
48. Kock, R.A. The Role of Wildlife in the Epidemiology of Rinderpest in East and Central Africa 1994–2004: A Study Based on Serological Surveillance and Disease Investigation. Ph.D. Thesis, University of Cambridge, Cambridge, UK, 2008.
49. Karimuribo, E.D.; Loomu, P.M.; Mellau, L.S.B.; Swai, E.S. Retrospective study on sero-epidemiology of peste des petits ruminants before its official confirmation in northern Tanzania in 2008. *Res. Opin. Anim. Vet. Sci.* **2010**, *1*, 184–187.
50. Swai, E.S.; Kapaga, A.; Kivaria, F.; Tinuga, D.; Joshua, G.; Sanka, P. Prevalence and distribution of Peste des petits ruminants virus antibodies in various districts of Tanzania. *Vet. Res. Commun.* **2009**, *33*, 927–936. [CrossRef] [PubMed]
51. Taylor, W.P.; Roeder, P.L.; Rweyemamu, M.M.; Melewas, J.N.; Majuva, P.; Kimaro, R.T.; Mollel, J.N.; Mtei, B.J.; Wambura, P.; Anderson, J.; et al. The control of rinderpest in Tanzania between 1997 and 1998. *Trop. Anim. Health Prod.* **2002**, *34*, 471–487. [CrossRef] [PubMed]
52. Diallo, A.; Okuthe, S.; Kamata, A. *Rapid Assessment for the Prevention and Control of Peste des Petits Ruminants, United Republic of Tanzania, Mission Report September 2010*; Crisis Management Centre Animal Health (CMC-AH), Food and Agriculture Organization of the United Nations (FAO) and World Organization for Animal Health (OIE), 2011; pp. 1–37. (Unpublished report).
53. Kivaria, F.M. Foot and mouth disease in Tanzania: An overview of its national status. *Vet. Q.* **2003**, *25*, 72–78. [CrossRef] [PubMed]
54. Mdetele, D.; Mwakabumbe, S.; Seth, M.; Madege, M. Evaluation of effectiveness of pest des petits ruminants vaccine in Northern Tanzania. *Res. Opin. Anim. Vet. Sci.* **2015**, *5*, 401–405.
55. Kgotlele, T.; Macha, E.S.; Kasanga, C.J.; Kusiluka, L.J.M.; Karimuribo, E.D.; Van Doorsselaere, J.; Wensman, J.J.; Munir, M.; Misinzo, G. Partial Genetic Characterization of Peste Des Petits Ruminants Virus from Goats in Northern and Eastern Tanzania. *Transbound. Emerg. Dis.* **2014**, *61*, 56–62. [CrossRef]
56. Jones, B.A.; Mahapatra, M.; Chubwa, C.; Clarke, B.; Batten, C.; Hicks, H.; Henstock, M.; Keyyu, J.; Kock, R.; Parida, S. Characterisation of Peste Des Petits Ruminants Disease in Pastoralist Flocks in Ngorongoro District of Northern Tanzania and Bluetongue Virus Co-Infection. *Viruses* **2020**, *12*, 389. [CrossRef]
57. Kgotlele, T.; Chota, A.; Chubwa, C.C.; Nyasebwa, O.; Lyimo, B.; Torsson, E.; Karimuribo, E.; Kasanga, C.J.; Wensman, J.J.; Misinzo, G.; et al. Detection of peste des petits ruminants and concurrent secondary diseases in sheep and goats in Ngorongoro district, Tanzania. *Comp. Haematol. Int.* **2019**, *28*, 755–759. [CrossRef]
58. Muse, E.A.; Karimuribo, E.D.; Gitao, G.C.; Misinzo, G.; Mellau, L.S.B.; Msoffe, P.L.M.; Swai, E.S.; Albano, M.O. Epidemiological investigation into the introduction and factors for spread of Peste des Petits Ruminants, southern Tanzania. *Onderstepoort J. Vet. Res.* **2012**, *79*, 6. [CrossRef]
59. Muse, E.A.; Matondo, R.B.; Karimuribo, E.D.; Misinzo, G.; Albano, M.O.; Gitao, G.C. Clinico-pathological findings of the 2011 outbreak of Peste des Petits (PPR) in Tandahimba district, southern Tanzania. *Res. Opin. Anim. Vet. Sci.* **2012**, *2*, 256–262.
60. Mbyuzi, A.O.; Komba, E.V.; Kimera, S.I.; Kambarage, D.M. Sero-prevalence and associated risk factors of peste des petits ruminants and contagious caprine pleuro-pneumonia in goats and sheep in the Southern Zone of Tanzania. *Prev. Vet. Med.* **2014**, *116*, 138–144. [CrossRef]
61. Kgotlele, T.; Kasanga, C.J.; Kusiluka, L.J.; Misinzo, G. Preliminary investigation on presence of peste des petits ruminants in Dakawa, Mvomero district, Morogoro region, Tanzania. *Onderstepoort J. Vet. Res.* **2014**, *81*, 3. [CrossRef] [PubMed]
62. Namtimba, A.M. Seroprevalence and Genetic Characterisation of Peste Des Petit Ruminants Virus in Selected areas of Tanzania. MSc Thesis, Sokoine University of Agriculture, Morogoro, Tanzania, 2015.
63. Swai, E.; Moshy, W.; Mbise, E.; Kaaya, J.; Bwanga, S. Serological evidence of camels exposure to peste des petits ruminants virus in Tanzania. *Res. Opin. Anim. Vet. Sci.* **2011**, *1*, 325–329.
64. Asil, R.M.; Ludlow, M.; Ballal, A.; Alsarraj, S.; Ali, W.H.; Mohamed, B.A.; Mutwakil, S.M.; Osman, N.A. First detection and genetic characterization of peste des petits ruminants virus from dorcas gazelles "Gazella dorcas" in the Sudan, 2016–2017. *Arch. Virol.* **2019**, *164*, 2537–2543. [CrossRef]
65. Dundon, W.G.; Diallo, A.; Cattoli, G. Peste des petits ruminants in Africa: A review of currently available molecular epidemiological data, 2020. *Arch. Virol.* **2020**, *165*, 2147–2163. [CrossRef]
66. Mdetele, D.; Seth, M.; Kabululu, M.; Misinzo, G.; Komba, E. A Comparative study of the Sero-prevalence of Peste Des Petits Ruminants Virus among Districts of Different Agro-Ecological Zones in Tanzania. *East Afr. J. Sci. Technol. Innov.* **2020**, *1*. [CrossRef]

67. George, J. Economic impact of Contagious Caprine Pleuropneumonia and Peste Des Petits Ruminants In Pastoral communities of Ngorongoro and Coastal Districts, Tanzania. MSc Thesis, Open University of Tanzania, Dar es Salaam, Tanzania, 2017.
68. Jones, B.A.; Rich, K.M.; Mariner, J.C.; Anderson, J.; Jeggo, M.; Thevasagayam, S.; Cai, Y.; Peters, A.R.; Roeder, P. The Economic Impact of Eradicating Peste des Petits Ruminants: A Benefit-Cost Analysis. *PLoS ONE* **2016**, *11*, e0149982. [CrossRef] [PubMed]
69. Kisoza, J. The role of local institutions in the management of agro-pastoral and pastoral systems: A case study of Mkata plains, Kilosa district and Ngorongoro Conservation Area, Ngorongoro district, Tanzania. MSc Thesis, The Sokoine University of Agriculture, Morogoro, Tanzania, 2007.
70. Mdetele, D.; Kassanga, C.; Seth, M.; Kayunze, K. Seroprevalence of foot and mouth disease in the wildlife-livestock interface and non-interface areas in Tanzania. *Res. Opin. Anim. Vet. Sci.* **2014**, *4*, 208–211.
71. Mwambene, P.L.; Mbwile, R.P.; Udo, H.F.; Kimbi, E.C.; Materu, J.; Mwaiganju, A.; Madoffe, S. Assessing dynamics of forced livestock movements, livelihoods and future development options for pastoralists/agro-pastoralists in Ruvuma and Lindi Regions, in the Southern Tanzania. *Livest. Res. Rural Dev.* **2014**, *26*. Available online: http://www.lrrd.org/lrrd26/1/mwam26004.htm (accessed on 14 June 2020).
72. Pingo's Formu; Land Rights Research and Resources Institute (HAKIARDHI). Report on Eviction and Resettlement of Pastoralists from Ihefu and Usangu-Mbarali District to Kilwa and Lindi Districts. 2008. Available online: https://www.tnrf.org/en/node/7180 (accessed on 25 June 2019).
73. Given, M.M.; Zebedayo, K.M.; Kizito, M.; Msomba, G.M.; Mvena, Z.K.; Mwajombe, K. Adaptive capacity of evicted agro-pastoralists from Ihefu Basin in Tanzania. *J. Agric. Ext. Rural Dev.* **2016**, *8*, 56–61. [CrossRef]
74. Munson, L.; Terio, K.A.; Kock, R.; Mlengeya, T.; Roelke, M.E.; Dubovi, E.; Summers, B.; Sinclair, A.R.E.; Packer, C. Climate Extremes Promote Fatal Co-Infections during Canine Distemper Epidemics in African Lions. *PLoS ONE* **2008**, *3*, e2545. [CrossRef] [PubMed]
75. Stermshorn, B.; Hargreaves, S.; Pacer, R.; Samake, F. PVS Gap Analysis: Preparation of a Plan to Strengthen the Veterinary Services of Tanzania. OIE Report—France, 2009. Available online: https://rr-africa.oie.int/en/our-mission/promotion-of-veterinary-services-improving-the-legal-framework-and-resources-of-national-veterinary-services/performance-of-veterinary-services-pvs-pathway-missions-status-of-implementation-in-africa/ (accessed on 21 June 2020).
76. Stermshorn, B.; Carron, M.; Munstermann, S.; Wakhusama, S. PVS Evaluation Follow-Up Mission Report. OIE Report France, 2016. Available online: https://rr-africa.oie.int/en/our-mission/promotion-of-veterinary-services-improving-the-legal-framework-and-resources-of-national-veterinary-services/performance-of-veterinary-services-pvs-pathway-missions-status-of-implementation-in-africa/ (accessed on 21 June 2020).

Published paper II

A Comparative study of the Sero-prevalences of Peste Des Petits Ruminants Virus among Districts of Different Agro-Ecological Zones in Tanzania

[1*] MDETELE D., [2]SETH M., [3]KABULULU M., [1]MISINZO G., [1]KOMBA E

[1]Southern African Centre for infectious diseases/SACIDS Foundation for one health, Sokoine University of Agriculture, P.O. Box 3015, [2]National Institute for Medical Research, Tanga Medical Research Centre, P. O. Box 5004, Tanga, Tanzania.

[3]Tanzania Livestock Research Institute – Uyole, P.O Box 6191, Mbeya, Tanzania

***Corresponding author**: daniel.mdetele@sacids.org

EISSN: 2707-0425

East African Journal of Science, Technology and Innovation, Vol. 1 (3): 2020.

A Comparative study of the Sero-prevalences of Peste Des Petits Ruminants Virus among Districts of Different Agro-Ecological Zones in Tanzania

[1*] MDETELE D., [2]SETH M., [3]KABULULU M., [1]MISINZO G., [1]KOMBA E

[1]Southern African Centre for infectious diseases/SACIDS Foundation for one health, Sokoine University of Agriculture, P.O. Box 3015,
[2]National Institute for Medical Research, Tanga Medical Research Centre, P. O. Box 5004, Tanga, Tanzania.
[3]Tanzania Livestock Research Institute – Uyole, P.O Box 6191, Mbeya, Tanzania
***Corresponding author**: daniel.mdetele@sacids.org

Abstract

Peste des petits Ruminants (PPR), a disease affecting sheep and goats, was confirmed in Tanzania in the year 2018. Since then the disease has continued to spread into different districts, causing significant socio-economic losses to livestock keepers. This study aimed at determining the sero-prevalence of PPR in 32 districts from the coastal, semi-arid and plateau ecological zones, respectively. Sera samples were collected from sheep and goats, and analysed by competitive Enzyme Linked Immunosorbent Assay (c-ELISA). Findings indicated that six (18.8%) districts had very high PPR sero-prevalence of which four (66.7%; Chamwino, Kondoa, Mvomero and Kilosa) belong to the semi-arid ecological zone and two (33.3%; Bagamoyo and Mkuranga) to the coastal ecological zone. Three districts (9.4%) had high PPR sero-prevalence, all from the semi-arid ecological zone. Twelve districts had low PPR sero-prevalence of which two (16.7%) were from semi-arid, one (8.3%) from coastal and nine (75.0%) from plateau ecological zones. A zero PPR sero-prevalence was recorded in three districts and eight districts from semi-arid and plateau ecological zones, respectively. There was a statistically significant difference in sero-positivity between the different ecological zones, $\chi 2\ (2) = 9.121$, $p = 0.010$, with a mean rank sero-positivity of 24.7% for coastal zone, 12.0% for plateau and 20.8% for semi-arid zone. *Post hoc* pairwise comparison with Bonferroni correction for multiple tests showed a statistically significant difference between plateau and semi-arid zones ($p = 0.032$). Although the coastal zone had a higher mean rank positivity than the plateau zone, the difference was not statistically significant ($p = 0.083$). The study suggests a zonal predisposition of PPR sero-prevalence with districts in the semi-arid and coastal zones having significantly higher values compared to those in the plateau ecological zones. Efforts for control of the disease need to concentrate in those two high risk ecological zones.

Keywords: *Ecological zones; PPR; Sero-prevalence; Tanzania*

Cite as: *Mdetele et al, 2020* ***A Comparative study of the Sero-prevalences of Peste Des Petits Ruminants Virus among Districts of Different Agro-Ecological Zones in Tanzania;*** *East African Journal of Science, Technology and Innovation 1(2)*

Received: 20/04/20
Accepted: 03/06/20
Published: 30/06/30

Introduction

Small ruminants play an important role in agricultural food production in developing countries (Devendra & McLeroy, 1982), including Tanzania (Shirima, 2005). In these countries, the sector also plays a crucial role in sustainable employment, contributing significantly to the economies and livelihoods of the smallholder farmers. However, the small ruminant industry in developing countries is constrained by factors such as high rates of infectious diseases as well as poor nutrition and marketing systems. Of the known infectious diseases, peste des petits ruminants (PPR) and contagious caprine pleuro-pneumonia (CCPP) are the most serious ones (Bölske et al., 1996; Malik et al., 2011; Rurangirwa et al., 1991); because of the associated high morbidity and mortality rates (Baron et al., 2016; Kipronoh et al., 2016; Singh, 2011). These two diseases pose a great threat to the livelihoods of many rural

small holder ruminant keepers, most of them being drawn from poor backgrounds (Diallo, 2006; El Hassan et al., 1984). Other important diseases include goat/sheep pox, contagious ecthyma, dermatophilosis and those caused by parasites (both internal and external).

Peste des petits ruminants (PPR) is a highly contagious and infectious viral disease of small ruminants (both domestic and wild) and camels (Khalafalla et al., 2010; Saeed et al., 2015; Zakian et al., 2016). It is an economically significant transboundary disease of sheep and goats causing significant losses among these animal populations. It is a major hindrance to small-ruminant production in areas where it occurs. The disease was first described in Côte d'Ivoire by Gargadennec and Lalanne in the year 1942. Currently, PPR is known to be present in a broad belt of sub-Saharan Africa, Arabia, the Middle East and Southern Asia. In these regions, the disease assumes an endemic pattern thereby causing severe impacts to the small ruminant industry. However, outbreaks of the disease have been reported in Turkey, China, Mongolia and Kazakhstan in recent years; indicating an expansion of its coverage resulting into a marked rise in the global incidence (Bao et al., 2011; Kock et al., 2015; Orynbayev et al., 2016; Shatar et al., 2017). The transboundary nature of the disease explains its potential for such an expansion and a rise in its incidence.

Primarily, PPR affects goats and sheep where it exhibits different levels of virulence, manifested by severity in the clinical presentation and time course of the disease. In most outbreaks of the disease, goats are severely affected while sheep are less severely affected, undergoing a mild form of the disease (Chauhan et al., 2009; Truong et al., 2014). In a few occasions, cattle and pigs can also be infected with the PPR virus (PPRV), although these animal species are not susceptible to clinical disease, and so infections in these animal species go without showing any symptoms (Anderson & McKay, 1994; Kumar et al., 2013; Sen et al., 2014; Taylor & Barrett, 1946). On the other hand, antelopes and other wild small ruminant species are severely affected by the PPRV, while the outcome of infection with the virus in other wild animal species is the development of antibodies without a clinical disease (Kinne et al., 2010; Mahapatra et al., 2015; Munir, 2014a; Orynbayev et al., 2016).

PPR is a contagious disease and therefore its transmission requires close contact between infected and susceptible animals. PPRV concentration is normally high in the exhaled air and body fluids such as oral and nasal discharges, urine and feces of the infected animals (Wernike et al., 2014). These discharges from the eyes, nose, mouth and faeces of an infected animal contain viruses, which are normally released into the environment when these affected animals cough, sneeze or defecate (Baron et al., 2014; Michael et al, 2015). Contact with those infectious materials is the most important way of transmission of the disease to healthy susceptible animals (Kihu et al., 2016; Couacy-Hymann et al., 2007; Fournié et al., 2018). PPRV is primarily transmitted via the respiratory route among animals living in close proximity. Thereafter, the virus spreads to other tissues of the body via regional lymph nodes, then infects the lymphocytes and infection spreads throughout the body via both the lymphatic and vascular systems. The virus is excreted by sick sheep and goats from one to two days prior to the development of clinical signs up to at least 10 days after the onset of signs in ocular, nasal and oral secretions at varying levels, and can be detected in faeces after the onset of signs for at least 10 days. PPRV does not survive long in the environment so indirect transmission plays a minor role and there is no evidence of a carrier state in infected animals.

Trade in live sheep and goats at markets where animals from different sources are brought into close contact increases chances for PPR transmission when the groups contain infected animals (Abubakar & Munir, 2014; Baron et al., 2017; Munir, 2014b; Swai et al., 2009). PPRV is now endemic in most of Africa and throughout Asia, where it is one of the main constraints to small ruminant production, trade and welfare. Therefore, it is a threat to food security and livelihoods of the poorest communities, to whom sheep and goats are often an important asset as most of the families can afford them as opposed to cattle (Baazizi et al., 2017; FAO & OIE, 2015). Moreover, spill-over of the PPRV from domestic animals to wildlife populations could result into serious concerns to the conservationists as it threatens the existence of endangered species (Kock et al., 2015; Munir, 2014a; Travis, Watson, & Tauer, 2011), thereby disturbing ecologies.

In Tanzania, several studies have been

conducted on PPR and results suggest that the disease is widespread in the country, thereby assuming endemicity. The studies are however fragmented and were not planned to provide a comprehensive picture of the country in relation to occurrence and distribution of the disease. Moreover, majority of these studies did not look into predictors for occurrence of the disease which is key in supporting endeavours to plan and develop disease control strategies. This in turn calls for more epidemiological studies which would cover aspects of analysis of risk factors responsible for occurrence and persistence of the disease among the rural farmer's goat and sheep populations.

Several studies have linked the occurrence and dynamics of infectious diseases in different animal populations to prevailing ecological features (Woma et al., 2016). The objective of this study was to estimate PPR sero-prevalence in selected districts from Tanzania, in relation to ecological zones in order to understand the variation in magnitude of the disease between the zones. Results of the study were intended to inform disease control strategies especially in helping to make rational decisions in allocation of limited resources by making use of the available epidemiological information.

Material and methods

Study areas

Tanzania is divided into eight different ecological zones that include the coastal, arid lands, semi-arid land, plateaux, Northern highlands, alluvial plains, Southern and Western highlands, with different physiological properties. Traditionally, in the past, there were areas for pastoralists, agro-pastoralists and crop producers in association with characteristics of these ecological zones to support their activities. Because of climate change which eventually resulted into shrinking of the grazing land, the importance of sheep and goats among pastoralists has increased significantly. As well, shifting of pastoralists and agro-pastoralists from their traditional territories has become inevitable.e. The current study therefore focused on three ecological zones in which small ruminants production is more prominent. Districts with large populations of goats and sheep were selected from the study zones, with the help of the District livestock offices in the respective zones. The Districts were selected from a list of those in which there had been no previous studies on the occurrence of PPR. Eventually, this study involved 32 districts of which three (9.4%), 12 (37.5%) and 17 (53.1%) were from the coast, semi-arid and plateau ecological zones, respectively.

Sampling procedure and sample collection

This study was carried out using stored serum samples collected from goats and sheep in the year 2013 under the United Nations Food and Agriculture Organization (FAO) project: Emergency assistance for early detection and control of PPR (PPR) TCP/URT/3302 (E). During implementation of the project, sampling was performed in 32 different districts in which PPR surveillance activities had never been carried out before, to assess if they have ever encountered the disease after the first outbreak of the disease in Tanzania in the year 2008. Staff in the respective Zonal Veterinary Investigation Centres (VICs) were involved in collection of the samples. The selection of Districts to be involved in the survey was purposively based on large populations of goats and sheep. Ten villages from each selected district and ten livestock keepers from each selected village were randomly selected for animal sampling. Five animals were selected for sampling from each selected herd using random procedures. From each animal approximately 5 ml of blood were collected from the jugular vein into a plain vacutainer tube. Blood samples were left to settle over night at room temperature before serum was harvested and stored into 2.5 ml cryovials at 4°C in the field and later at respective VIC at -20°C before shipping to Sokoine University of Agriculture (SUA) for laboratory analysis. At SUA the samples were stored at -80°C freezers up to the time of laboratory analysis.

Laboratory analysis of the collected serum samples

The detection of antibodies to PPRV from the collected serum samples was done using c-ELISA. The test is based on the competition between the anti-PPR monoclonal antibodies and antibodies in the serum sample binding to the PPR antigen. The presence of antibodies to PPRV serum sample outcompete and block reactivity of the monoclonal antibody resulting in the reduction in expected colour (signal) following the addition of enzyme labelled anti-mouse conjugate and substrate or chromogen solution. The assay was performed according to the general principle of c-ELISA as previously

described by Anderson & McKay (1994). Briefly, Nunc Maxisorb 96-well microtitre plates were used for all the assays. Volumes of 50 ul of serum were used throughout the test after thawing. The PPR antigen was adsorbed to the plate using phosphate buffered saline (PBS), and all the other reagents were added in blocking buffer (PBS supplemented with 0-1% [v/v] Tween-20 and 0.3% [v/v] normal bovine serum). All incubation steps were for 1 h at 37°C on an orbital shaker. Plates were washed three times after each incubation step by flooding them with PBS then emptying. Mouse immunoglobulin was detected using rabbit anti-mouse immunoglobulin conjugated to horseradish peroxidase (HRPO). Hydrogen peroxide/orthophenylene diamine (OPD) was used as a substrate/chromogen. Samples with percentage inhibition values of more than 50% were considered positive.

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS®) software version 21. Descriptive statistics were computed to determine sero-prevalences for districts in respective ecological zones. A non-parametric test - Kruskal Wallis H was used to determine whether there existed a statistically significant difference in district-level sero-prevalence between the three ecological zones. *Post hoc* pairwise comparisons were performed, with Bonferroni correction for multiple comparisons, to indicate which ecological zones differed in sero-prevalence. For the purpose of this study sero-prevalences of PPR at district level were categorized as very high (>20%), high (11-20%) and low (1-10%). Quantum Geographical Information System (QGIS) was used to create a map displaying the 32 districts involved in this study.

Results

The study involved 32 districts of which three (9.4%) were from coast ecological zone, 12 (37.5%) from the semi-arid ecological zone and 17 (53.1%) from the plateau ecological zone. The mean rank sero-positivity of the three ecological zones are shown in figure 2. A total of 2,490 serum samples were analysed and the overall sero-positivity across all districts was 20.1%. The study showed that six (18.8%) districts had very high PPR sero-prevalences of which four (66.7%) belonged to the semi-arid ecological zone (Chamwino, Kondoa, Mvomero and Kilosa) and two (33.3%) were from the coastal ecological zone (Bagamoyo and Mkuranga). Three districts (9.4%; Shinyanga, Meatu and Iramba) had high PPR sero-prevalence, all from the semi-arid ecological zone. Magnitudes of PPR sero-prevalences for other districts are shown in table 1. Kruskal-Wallis H test showed that there was a statistically significant difference in sero-positivity between the three ecological zones, $\chi 2\ (2) = 9.121$, $p = 0.010$, with a mean rank sero-positivity of 24.7% for coastal zone, 12.0% for plateaux and 20.8% for semiarid zone. *Post hoc* pairwise comparisons showed a statistically significant difference in sero-prevalence between plateau and semi-arid ecological zones ($p = 0.032$). Although the coastal ecological zone had a higher mean rank sero-positivity than the plateau ecological zone, there was no statistically significant difference between them ($p = 0.083$). Likewise, no statistically significant difference was found in sero-positivity between the coastal and semi-arid ecological zones ($p = 0.518$).

Table 1: PPR sero-prevalence (%) and sero-prevalence magnitude in study districts and ecological

zones

District	Seropositivity (%)	Level	Ecological zone	Production system
Mvomero	75	Very high	Semi-arid	PastoAgropasto
Kilosa	63.8	Very high	Semi-arid	PastoAgropasto
Bagamoyo	35.2	Very high	Coast	PastoAgropasto
Mkuranga	47.8	Very high	Coast	AgroPasto
Kisarawe	4.4	Low	Coast	AgroPasto
Misenyi	5	Low	plateaux	AgroPasto
Karagwe	0	Zero	plateaux	AgroPasto
Ngara	2.8	Low	plateaux	AgroPasto
Kahama	3.5	Low	Semi-arid	AgroPasto
Shinyanga	10.7	High	Semi-arid	AgroPasto
Maswa	0	Zero	Semi-arid	AgroPasto
Meatu	15.2	High	Semi-arid	AgroPasto
Ukerewe	0	Zero	Plateaux	AgroPasto
Sengerema	9.8	Low	Plateaux	AgroPasto
Misungwi	5	Low	Plateaux	AgroPasto
Kwimba	0	Zero	Plateaux	AgroPasto
Magu	1.9	Low	plateaux	AgroPasto
Ilemela	3.8	Low	plateaux	AgroPasto
Nyamagana	2	Low	plateaux	AgroPasto
Chamwino	53.5	Very high	Semi-arid	AgroPasto
Kondoa	33.5	Very high	Semi-arid	AgroPasto
Iramba	14.1	High	Semi-arid	AgroPasto
Singida	7.3	Low	Semi-arid	AgroPasto
Kigoma rural	0	Zero	Plateaux	AgroPasto
Kasulu	0	Zero	Plateaux	AgroPasto
Kibondo	0	Zero	Plateaux	AgroPasto
Igunga	0	Zero	Semi-arid	AgroPasto
Nzega	0	Zero	Semi-arid	AgroPasto
Tabora	5	Low	Plateaux	AgroPasto
Nsimbo	5.9	Low	Plateaux	AgroPasto
Mpanda	0	Zero	Plateaux	AgroPasto
Mlele	0	Zero	Plateaux	AgroPasto

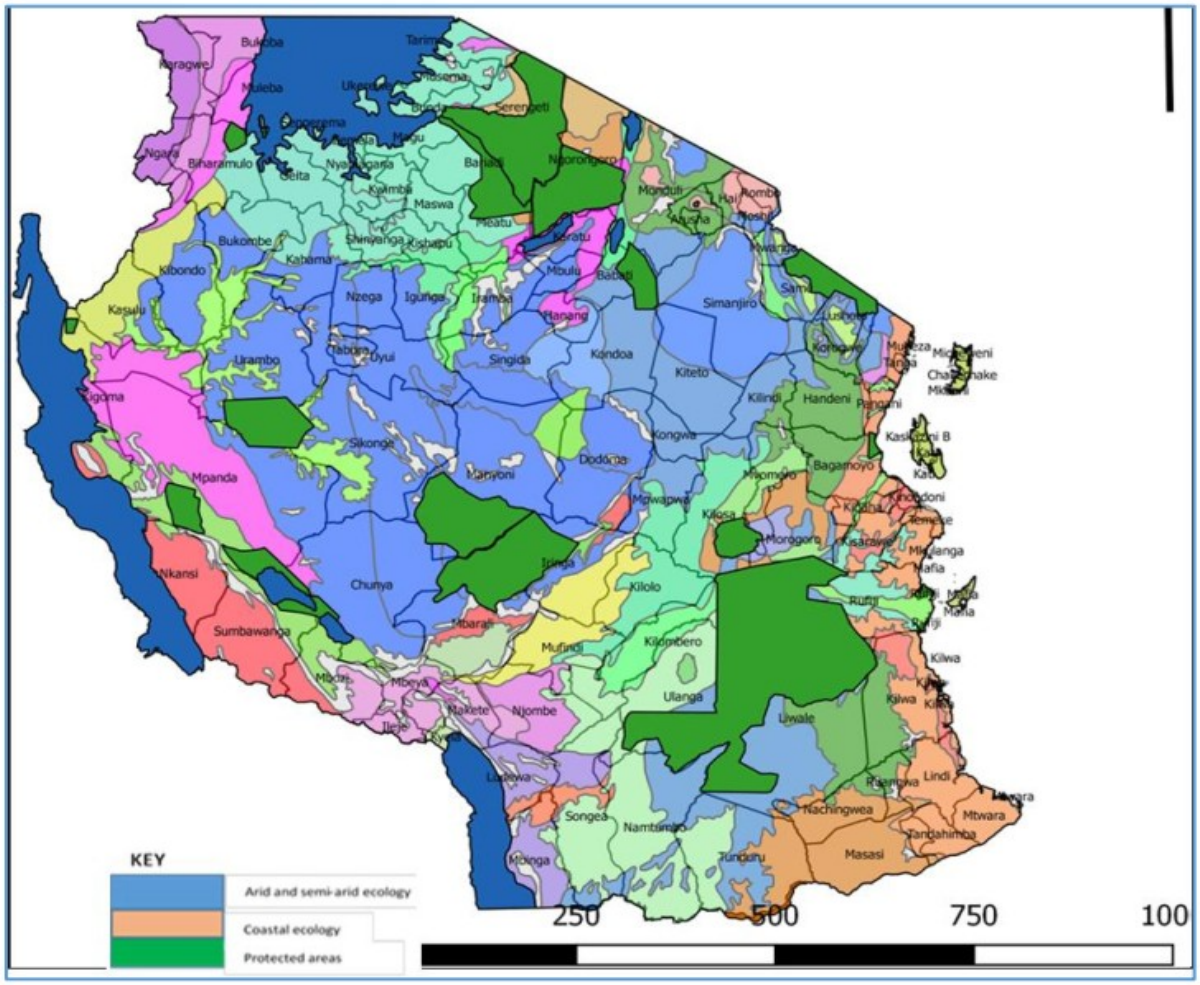


Figure 1: Tanzania map showing districts with respective ecological zones

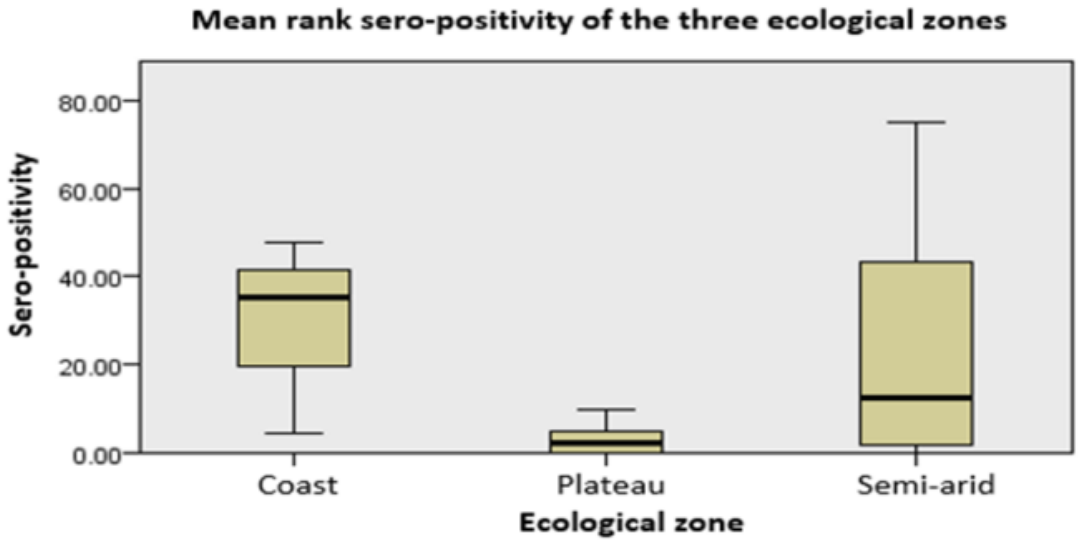


Figure 2: Mean rank sero-positivity of the three ecological zones

Discussion

The present study was aimed at determining the differences in sero-prevalence of PPR in districts from different agro-ecological zones of Tanzania with unknown disease status. Samples were collected in areas where PPR surveillance activities had never been carried out before since confirmation of the disease in Tanzania in the year 2008. Analysis of 2,490 samples from sheep and goats confirmed the endemic nature and wide distribution of PPR throughout Tanzania. Results have shown that PPR sero-prevalence in the country is very high in magnitude in the semi-arid and coastal ecological zones. These findings are in line with what has been reported in previous studies carried out in the country, where PPR sero-prevalence were found to be higher in arid and semi-arid districts of Ngorongoro, Longido, Simanjiro and Monduli (Kivaria et al., 2014; Swai et al., 2009), as well as coastal districts of Lindi and Mtwara (Franklin et al., 2015; Kivaria et al., 2014; Muse et al., 2012). Similarly, in other parts of the East African region, studies have shown PPR sero-prevalences to be higher in arid and semi-arid areas. This includes studies conducted in Turkana and Karamoja in Kenya and Uganda, respectively (Nkamwesiga et al., 2019; Dundon et al., 2017; Gitao et al., 2014; Kihu et al., 2015; Ruhweza et al., 2010). In Nigeria, sero-survey of PPRV in small ruminants from different ecological zones showed that the states found on savannah areas, which are ecologically similar to arid and semi-arid zones, had higher PPR sero-prevalence compared to states in the tropical rain forest and plateau (Woma et al., 2016). According to Lefèvre and Diallo (1990), PPRV infections persist in regions of low relative humidity, and that the PPRV survives longer in dry regions (Morandell et al., 2008). The mentioned attributes are a characteristic of the zones found to have higher sero-prevalence in this study, and therefore their observations are in support of the findings of the current study.

Over the years, arid and semi-arid areas in Tanzania have been a home for pastoralists. These areas have as well been preferred by wildlife, and also used in establishment of conserved areas such as national parks, game controlled areas or wildlife management areas (Kideghesho & Msuya, 2012). This is because these areas are good at having animal feed resources like pasture, water and mineral licks in the soil as they have been conserved for a long time (Kideghesho et al., 2013). Since the areas are not suitable for crop farming, livestock keepers, especially pastoralists from different places take their animals for grazing on such areas. They sometimes take their animals to the boundaries of conserved areas and even inside particularly during the dry period when looking for pasture, water and mineral licks (Kideghesho & Msuya, 2012). In so doing, livestock from different places and herdsare gathered on the area, faciliting transmission of infectious diseases among the animals. This partly supports the observation in this study on

higher prevalence of PPR in these areas as opposed to other ecological zones where animal populations are fairly small and interactions between animals from different places and herds are seldom. Hence, minimizing contact between animals from different places and herds could be considered in attempts to control this devastating disease. Moreover, PPR investigation in wildlife mingling with small ruminants in the protected and interface areas is necessary because the role of wildlife in the epidemiology of PPR is not well established which presents a gap in understanding the epidemiology of the disease. If the disease spills over to wildlife and is maintained and circulates among wildlife species it will be much more difficult to control than when it is only in the livestock population which is easy to manage.

Sheep and goat production is gaining its importance among pastoralists as grazing and watering areas are shrinking in their territories, because of climate change and changes of land use policy among different stakeholders. Such a change of situation is forcing pastoralists to shift from cattle production, which has higher demands for pasture and water to small ruminants, which are hardy, and have less demands for pasture and water. In some occasions, due to climate change, livestock keepers are forced to leave areas known to be their territories in arid and semi-arid areas to other areas looking for water and pasture. Since 2006 many pastoralists have been moving to coastal areas because of availability of grazing land. The influx of livestock from different places into the coastal zone, which was not previously used for livestock production, could be responsible for PPR introduction into the area. This is exacerbated by absence of animal health checks as the animals get into these new areas due to several reasons which include shortage of workforce as well as inadequate enforcement of laws that are in support of disease control. Eventually, following introduction of the disease, its further spread was facilitated through animal interactions which are highly supported by the prevailing extensive animal husbandry system, hence the observed high PPR sero-prevalence in the zone. Animal movements and interactions result into considerable changes in the epidemiological patterns of not only PPR but also of other infectious diseases.

Our findings point out to a relatively lower sero-prevalence of PPR in ecological zones characterized by a more humid environment as compared to those featured by less humid environments.These areas are favourable for crop production and therefore communities are mainly involved in crop production. Consequently, these areas have very few livestock, including goats and sheep, and hence limited chances for contacts between animals from different herds. This makes chances of PPR transmission in these areas to be minimal compared to the arid and semi-arid areas which are preferred by pastoralists and agro-pastoralists due to lower level of crop production activities, which also prevents occurrence of conflicts between crop producers and livestock keepers. Thus, the lower livestock numbers in the humid environments, mostly used in crop production, could partly account for the lower sero-prevalence of PPR in the ecological zones.

Results of the present study may have been biased by the sampling method employed and the small sample size adopted. As the selection of zones was based on higher sheep and goat populations, and lack of previous studies on the epidemiology of PPR, this resulted into inadequate coverage as eventually only three ecological zones were involved. These criteria prevented obtaining useful information from other zones which were not involved in the study, that could lead to a fair comparison and provide a more comprehensive picture of the distribution of the disease in the country. This limitation could however be addressed by the use of the existing information to build spatial and temporal models that could be used in a cost-effective manner to determine the presence and extent of the disease in other zones for comparison purposes.

A total of 32 districts were investigated for the occurrence and extent of PPR infections among goat populations in the current study. All these were the districts in which there was no prior knowledge in their status with respect to PPR infections in the goat populations. Out of the studied districts, about two thirds (65.6%) turned out to be positive for the disease. Six of the positive districts (18.8%) had very high PPR sero-prevalence whereas three of them (9.4%) had high PPR sero-prevalence. This finding is worth noting as it implies that the PPRV is widespread among goat populations in the country, as data are available for occurrence of

the disease in many other districts across the country. This should present a wake-up call among authorities responsible for animal health as well as other stakeholders in the livestock sector that concerted efforts are required to combat livestock diseases especially those of significant socio-economic impacts to small holder farming communities. Activities focusing on preventing disease spread through breaking the weakest links in the transmission cycles should be given due priority. This imply changes in the animal husbandry systems and strengthening animal health service delivery by reaching rural farmers.

An important finding in the present study was a zero PPR sero-prevalence in three districts in the semi-arid zone and eight districts in the plateau ecological zone. From the disease control point of view, this is good news as these districts could be considered in the formation of disease free zones which could be considered in the production of disease free small ruminants for the export market. This technique has been adopted elsewhere in which zoning has been opted in the production of disease free animals for export so as to be able to reach the export market which is featured by stringent conditions including requiring exporting countries to be free from transboundary animal diseases. This would however require an additional work of screening the small ruminant populations in these districts for other equally important transboundary animal diseases which present an obstacle in reaching the export market. Generally, the control and eradication of PPR and other economically important small ruminant diseases is vital in order to alleviate poverty and improve the health and husbandry of small ruminant populations kept by people in resource limited settings.

Conclusions and Recommendations

The occurrence of PPR in the country shows ecological zone pre-disposition, with sero-prevalences being higher in semi-arid and coastal zones characterized by low relative humidity. This is suggestive of the prevalence of favourable conditions for survival and perpetuation of the virus among reservoir hosts and the susceptible goat populations in these areas. Control or eradication strategies for the disease should consider putting more efforts in these high risk areas as determined by the disease prevalence rates. The aim is to enhance informed decision making on disease control strategy.

Acknowledgements

The study was supported by the United Nations Food and Agriculture Organization (FAO) project: Emergency assistance for early detection and control of PPR (PPR) TCP/URT/3302 (E), the Tanzanian government through the Ministry of Livestock and Fisheries, and The Southern African Centre for Infectious Disease (SACIDs) of Sokoine University of Agriculture (SUA) which are all highly thanked for their support. Moreover, my supervisors from SUA and the Royal Veterinary College - London are gratefully acknowledged. The Global Challenge relief fund (GCRF) is acknowledged for financial support.

References

Abubakar, M., & Munir, M. (2014). Peste des Petits Ruminants virus: An emerging threat to goat farming in Pakistan. *Transboundary and Emerging Diseases*, *61*(SUPPL1.), 7–10. https://doi.org/10.1111/tbed.12192

Anderson, J., & McKay, J. A. (1994). The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implications to rinderpest control programmes. *Epidemiology and Infection*, *112*(1), 225–231. https://doi.org/10.1017/S0950268800057599

Baazizi, R., Mahapatra, M., Clarke, B. D., Ait-Oudhia, K., Khelef, D., & Parida, S. (2017). Peste des petits ruminants (PPR): A neglected tropical disease in maghreb region of North Africa and its threat to Europe. *PLoS ONE*, *12*(4). https://doi.org/10.1371/journal.pone.0175461

Bao, J., Wang, Z., Li, L., Wu, X., Sang, P., Wu, G., … Qi, L. (2011). Detection and genetic characterization of peste des petits ruminants virus in free-living bharals (Pseudois nayaur) in Tibet, China. *Research in Veterinary Science*. https://doi.org/10.1016/j.rvsc.2010.05.031

Baron, J., Bin-Tarif, A., Herbert, R., Frost, L., Taylor, G., & Baron, M. D. (2014). Early

changes in cytokine expression in peste des petits ruminants disease. *Veterinary Research*, *45*(1). https://doi.org/10.1186/1297-9716-45-22

Baron, M. D., Diallo, A., Lancelot, R., & Libeau, G. (2016). Peste des Petits Ruminants Virus. In *Advances in Virus Research*. https://doi.org/10.1016/bs.aivir.2016.02.001

Baron, Michael D. (2015). The molecular biology of peste des petits ruminants virus. In *Peste Des Petits Ruminants Virus*. https://doi.org/10.1007/978-3-662-45165-6_2

Baron, Michael D., Diop, B., Njeumi, F., Willett, B. J., & Bailey, D. (2017). Future research to underpin successful peste des petits ruminants virus (PPRV) eradication. *Journal of General Virology*. https://doi.org/10.1099/jgv.0.000944

Bölske, G., Mattsson, J. G., Bascunana, C. R., Bergström, K., Wesonga, H., & Johansson, K. E. (1996). Diagnosis of contagious caprine pleuropneumonia by detection and identification of Mycoplasma capricolum subsp. capripneumoniae by PCR and restriction enzyme analysis. *Journal of Clinical Microbiology*. https://doi.org/10.1128/jcm.34.4.785-791.1996

Cg, G., Kihu S, & Sm, M. (2016). Review of Peste Des Petits Ruminants in Sheep. *J Vet Med Res*.

Chauhan, H. C., Chandel, B. S., Kher, H. N., Dadawala, A. I., & Agrawal, S. M. (2009). Pesti des petits ruminants virus infection in animals. *Veterinary World*. https://doi.org/10.5455/vetworld.2009.150-155

Couacy-Hymann, E., Bodjo, S. C., Danho, T., Koffi, M. Y., Libeau, G., & Diallo, A. (2007). Early detection of viral excretion from experimentally infected goats with peste-des-petits ruminants virus. *Preventive Veterinary Medicine*, *78*(1), 85–88. https://doi.org/10.1016/j.prevetmed.2006.09.003

Devendra, C., & McLeroy, G. B. (1982). Goat and sheep production in the Tropics. *Goat and Sheep Production in the Tropics*. https://doi.org/10.1016/0921-4488(94)90018-3

Diallo, A. (2006). Control of peste des petits ruminants and poverty alleviation? *Journal of Veterinary Medicine Series B: Infectious Diseases and Veterinary Public Health*. https://doi.org/10.1111/j.1439-0450.2006.01012.x

Dundon, W. G., Kihu, S. M., Gitao, G. C., Bebora, L. C., John, N. M., Oyugi, J. O., … Diallo, A. (2017). Detection and Genome Analysis of a Lineage III Peste Des Petits Ruminants Virus in Kenya in 2011. *Transboundary and Emerging Diseases*, *64*(2), 644–650. https://doi.org/10.1111/tbed.12374

El Hassan, S. M., Harbi, M. S. M. A., & Bakr, M. I. A. (1984). Treatment of contagious caprine pleuropneumonia. *Veterinary Research Communications*. https://doi.org/10.1007/BF02214697

FAO, & OIE. (2015). *Global strategy for the control and eradication of PPR. International conference for the control and eradication of Peste des Petits Ruminants (PPR). Abidjan, Ivory Coast.*

Fournié, G., Waret-Szkuta, A., Camacho, A., Yigezu, L. M., Pfeiffer, D. U., & Roger, F. (2018). A dynamic model of transmission and elimination of peste des petits ruminants in Ethiopia. *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.1711646115

Franklin, S. &, Misinzo, G., Kgotlele, T., Muse, E. A., Doorsselaere, J. Van, Berg, M., & Munir, M. (2015). Short Communication. *British Journal of Virology*, *2*(1).

Gitao CG, Ithinji DG, Gitari R, & Ireri GR. (2014). The confirmation of Peste des petit ruminants (PPR) in Kenya and perception of the disease in West Pokot. *Res. Opin. Anim. Vet. Sci*, *4*(6), 312–317. Retrieved from http://www.roavs.com/pdf-files/Issue-6-2014/312-317.pdf

Khalafalla, A. I., Saeed, I. K., Ali, Y. H., Abdurrahman, M. B., Kwiatek, O., Libeau, G., … Abbas, Z. (2010). An outbreak of peste des petits ruminants (PPR) in camels in the Sudan. *Acta Tropica*, *116*(2), 161–165. https://doi.org/10.1016/j.actatropica.2010.08.002

Kideghesho, J. R., & Msuya, T. S. (2012). Managing the Wildlife Protected Areas in the Face of Global Economic Recession , HIV / AIDS Pandemic , Political Instability and Climate Change : Experience of Tanzania.

Kideghesho, J. R., Rija, A. A., Mwamende, K. A., & Selemani, I. S. (2013). Emerging issues and challenges in conservation of biodiversity in the rangelands of Tanzania. *Nature Conservation*, *6*(November), 1–29. https://doi.org/10.3897/natureconservat

Manuscript III

Characterization of Peste des Petits Ruminants outbreaks in the Serengeti Ecosystem

Mdetele, D.[1,2*] Nyasebwa, O[7], Kabululu. M[6], Makange, M[2]. Benfield, C[4]. Mahapatra, M[5]. Parida, S.[5] Kock, R.[4] Misinzo, G.[2] Komba, E.[3] and Jones, B.[4]

[1]Ministry of Livestock and Fisheries, Directorate of Veterinary Services, P.O Box 2870 Mji wa Serikali, Dodoma, Tanzania

[2]Department of Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical sciences, P.O. Box 3019, Chuo Kikuu, Morogoro, Tanzania

[3]Department of Medicine, Collage of Veterinary Medicine and Biomedical sciences, P.O. Box 3021, Chuo Kikuu.

[4]Royal Veterinary College, Hawkshead Lane, Hatfield, Herts, AL9 7TA, UK

[5]The Pirbright Institute, Ash road, pirbright working surrey, GU24ONF, UK

[6]Tanzania Livestock Research Institute (TALIRI) - Uyole, P.O. Box 6191, Mbeya, Tanzania.

[7]Ministry of livestock and Fisheries, Veterinary council of Tanzania, P.O Box 2870, Dodoma, Tanzania.

*Submitted to animal journal

2.3 Characterization of Peste des Petits Ruminants outbreaks in the Serengeti Ecosystem

Mdetele, D.[1,2*] Nyasebwa, O[7], Kabululu. M[6], Makange, M[2]. Benfield, C[4]. Mahapatra, M[5]. Parida, S.[5] Kock, R.[4] Misinzo, G.[2] Komba, E.[3] and Jones, B.[4]

[1]Ministry of Livestock and Fisheries, Directorate of Veterinary Services, P.O Box 2870 Mji wa Serikali, Dodoma, Tanzania

[2]Department of Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical sciences, P.O. Box 3019, Chuo Kikuu, Morogoro, Tanzania

[3]Department of Medicine, Collage of Veterinary Medicine and Biomedical sciences, P.O. Box 3021, Chuo Kikuu.

[4]Royal Veterinary College, Hawkshead Lane, Hatfield, Herts, AL9 7TA, UK

[5]The Pirbright Institute, Ash road, pirbright working surrey, GU24ONF, UK

[6]Tanzania Livestock Research Institute (TALIRI) - Uyole, P.O. Box 6191, Mbeya, Tanzania.

[7]Ministry of livestock and Fisheries, Veterinary council of Tanzania, P.O Box 2870, Dodoma, Tanzania.

Abstract

Peste des petits ruminants (PPR) is a severe transboundary animal disease of sheep and goats commonly found in East Africa. The disease has been reported in camels and other artiodactyl wildlife species elsewhere. It is transmitted through direct and indirect contact among infected and health animals. The study aimed at characterizing the epidemiology of PPR in a complex ecosystem with multiple livestock and wildlife species sharing resources between countries in the cross border communities found in Karatu, Longido,

Meatu, Monduli, Ngorongoro and Serengeti districts of Tanzania. Data collection involved outbreak investigation, household interviews and herd dynamics examination techniques. The samples collected during outbreak investigation were analysed by lateral flow device, c-ELISA and qPCR tests. A total of 78 flocks were examined in Karatu (n=10), Longido (n=9), Meatu (n=7), Monduli (n=11), NCAA (n=13), Ngorongoro (n=18) and Serengeti (n=10). Out of six study districts, PPR was confirmed in four districts namely; Karatu, Longido, Ngorongoro and Serengeti. In Ngorongoro, PPR was confirmed in all the three divisions of the district (Loliondo, Sale and NCAA), while in Karatu district, PPR was confirmed in Mang'ola, the ward bordering with NCAA. In the divisions of Ngorongoro district PPR was confirmed in the following wards: Loliondo (Oloipiri, Arash, Soitsambu and Enguserosambu), NCAA (Olbalbal) and Sale (Samunge and Malambo). In Longido confirmed in Engarenaibor and Sinya wards. Peste des Petits ruminant virus lineage III was found to be the predominant lineage circulating in the area. The confirmed cases of PPR were higher among young sheep and goats coexisting with wildlife and those found in Tanzania and Kenya border wards. The disease presented itself with mild clinical signs unlike in a naïve population. The pastoralist communities were found to be aware of the disease clinical signs. Occurrence of PPR in livestock populations co-existing with wildlife and those raised by border communities threatens spill over of disease to endangered wildlife and spread to the neighbouring countries, respectively. This necessitates collaboration between veterinary and conservation authorities and between neighbouring countries on surveillance and Control of PPR.

Key words: PPR, Epidemiology, Ecosystem, Livestock and wildlife

Introduction

Peste des petits ruminants (PPR) is a severe disease of sheep and goats, commonly occurring in East Africa. As well, it affects camels and other artiodactyl wildlife species (Munir, 2014; Aziz-ul-Rahman *et al.,* 2018). In livestock, the disease is characterized by high morbidity and mortality, causing loss of milk and meat, threatening food security and livelihoods of pastoralists and smallholder farmers. Spill-over of the PPRV from domestic to wildlife populations could result into serious concerns to the conservationists as it threatens the existence of endangered species (Travis *et al.,* 2011; Munir, 2014; Kock *et al.,* 2015) thereby disturbing ecologies. The causative agent, PPR virus (PPRV), is a morbillivirus belonging to the family *Paramyxoviridae* sharing genetic and antigenic characteristics with rinderpest (RP) virus (Diallo *et al.,* 2007; Kumar *et al.,* 2014; Munir *et al.,* 2012; Rahman *et al.,* 2018). PPR is a highly transmissible transboundary animal disease, with potentially devastating impacts on small ruminants, as morbidity and mortality rates can be up to 100% in naive populations (Abu Elzein *et al.,* 2004; Kumar *et al.,* 2014; Sharma *et al.,* 2015). From natural and experimental research it has been shown that cattle in close proximity to infected goat and sheep can be infected with PPRV, although they do not appear to be susceptible to clinical disease. It has been reported that cattle and pigs become sub-clinically infected (Taylor and Barrett, 1946; Anderson and McKay, 1994; Kumar *et al.*, 2013; Sen *et al.,* 2014), but recent experimental work has shown that pigs can be clinically infected and transmit PPRV (Schulz *et al.,* 2018). PPR for first time was suspected in the northern pastoral areas of Tanzania towards the end of the year 2008 (Swai *et al.,* 2009). The disease was confirmed and officially reported to the World Organization for Animal Health (OIE) on the 27th of January 2009. By the end of 2010 the disease had further spread to eastern and southern parts of the country (Muse *et al.,* 2012; Kivaria et *al.,* 2014).

Pest des petits ruminant virus (PPRV) infected animal transmit the virus to healthy animals through the respiratory route. The virus is shed by sick sheep and goats prior to the development of clinical signs and up to 10 days after the onset of signs (Truong *et al.,* 2014). The virus concentration is high in the excretions such as saliva, oral/nasal discharges, urine and faeces of the infected animals (Parida *et al.,* 2015). Contact with those infectious materials is the most important way of transmission of the disease to healthy susceptible animals. In severe cases that involve naive populations, the clinical disease is usually acute, with pyrexia of 41 to 42°C and several animals in the flock get infected at the same time (Truong *et al.,* 2014).

Live animal trade especially of sheep and goats at markets where animals from different sources are brought together into close contact, mixing infected and naïve animals, increases chances for PPR transmission (Swai *et al.,* 2009; Abubakar and Munir, 2014; Munir, 2014; Baron *et al.,* 2017). Grazing systems, watering points and use of common markets and slaughter points link animals from different production systems and become the focal for epidemic spreading (Swai *et al.,* 2011; Abubakar and Munir, 2014). Traders collectively facilitate the contact networks that provide opportunities for disease transmission. The long distance movement of livestock during drought periods increases chances of interaction between livestock and wildlife at grazing and watering points (Mdetele *et al.,* 2014) all these can facilitate spread and maintenance of PPRV.

Of recent, there have been efforts at international and national levels, aiming at eradicating PPR globally. Since the emergence of the disease in Tanzania, there have been vaccination campaigns to limit its impact on livestock keepers, yet outbreaks continue to occur. One of the main challenges is lack of effective surveillance systems that would guide allocation of the limited resources based on perceived risk profiles. Vaccination is usually applied in

response to outbreaks based on funds availability, aiming to reduce livestock keepers' immediate losses due to the disease. However, low levels of vaccination coverage could be contributing to persistence and maintenance of the virus in the country. A more pragmatic, research driven control programme is warranted to halt persistence and spread of the PPR in Tanzania, as well as other countries endemic for the disease.

The broader objective of this study was to establish the epidemiology of PPR in the livestock populations in the greater Serengeti ecosystem. Specifically the study aimed at; i) looking at the interaction of wildlife and livestock and how the multi-host system influenced the epidemiology of PPR in comparison to a single host system, ii) determining the role of wildlife in the maintenance and spread of PPRV in the greater Serengeti area.

This work build on the previous studies by Jones *et al.* (2020) and Mahapatra *et al.* (2015), which came up with the following findings; i) there is clinical variability in PPR cases, which makes the field diagnosis of PPR challenging, highlighting the importance of access to pen-side antigen tests and multiplex assays to support improved surveillance, ii) free-ranging wildlife are susceptible to infection and can act as sentinels of livestock disease but do not appear to be maintaining infection across their populations. Yet it is not known whether wildlife is becoming infected by contact with sheep and goats, or the virus is circulating independently among wildlife. Compared to the previous studies, this study was done in an extended area of six districts and much longer period of eighteen months.

Methodology

Study area

The study was carried out in Serengeti ecosystem comprising of Karatu, Longido, Meatu, Monduli, Ngorongoro and Serengeti districts of the northern zone of Tanzania (Figure 1).

The zone is characterized by having large number of wildlife interacting with livestock in the protected areas found in those districts. Serengeti district is found on the western side of the ecosystem and comprises of Serengeti National park, Ikorongo and Grumeti game reserves as protected areas. Meatu district is in the south west of the ecosystem and comprises of Maswa and Makao game reserves. Karatu district is on the south east of the ecosystem bordering with NCAA. Monduli and Longido districts are found on the eastern side of the ecosystem, these have wildlife in areas set as wildlife management areas and corridors. Ngorongoro district is in the epicentre of the ecosystem composing of Ngorongoro Conseravation Area Authority (NCAA) and Loliondo game controlled area (LGCA) which are multiple land use areas. The area is a home to famous pastoralists in East Africa i.e. the Maasai, barbaig (Mang'ati/Datooga) and agropastoralists belonging to Sukuma, Kurya and iraqw (Wamburu) ethnic groups. These ethnic groups are famous in cattle, goat and sheep production in Tanzania. Their livestock in some areas graze with wildlife from the conserved areas which mostly include Zebra, Thomson's gazelles, Impala, Grant's gazelles, Topi, buffaloes and giraffe. The study included sampling and data collection that was done in a period of eighteen months from January 2018 to July 2019.

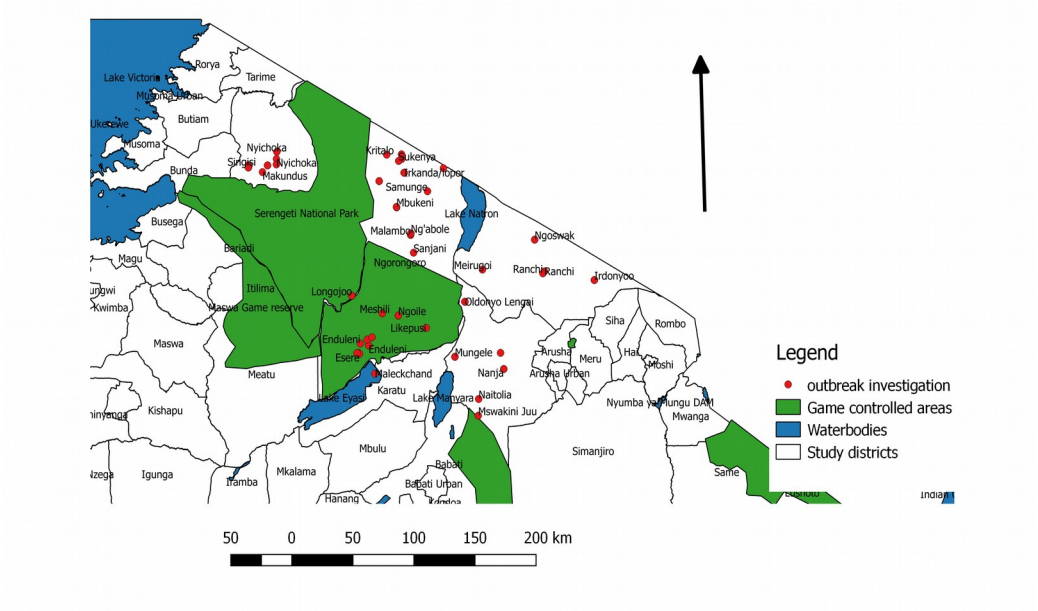


Figure 1: Map of northern Tanzania showing greater Serengeti ecosystem Villages where outbreak investigation was carried out during the study period

Data collection

Field data gathering

The method used in the study was adopted from the previous study by Jones *et al.* (2020) especially in outbreak investigation with some modifications. All the study districts were visited to collect baseline information about production system, ethnic groups, wildlife livestock interactions, livestock movements, animal health staffing/administrative structure, common diseases, animal health services/facilities available, PPR vaccination history and livestock marketing (File S1).

PPR and PPR like disease outbreaks reported by livestock field officers (LFOs) and district veterinary officers (DVOs) before and during the study period in all the study districts were investigated. Flocks were selected and examined during investigation. In total 78 flocks were investigated in all the six study districts. Examined cases mostly had

either one or all of the following: high mortality, ocular/nasal discharges, mouth lesions, respiratory signs and diarrhoea in sheep and goats. The visited herds with reported cases were either at household or at grazing. While doing investigation, semi-structured interviews were conducted to the family member found during the visit. As well, interviews were extended to other villagers in case they were available and willing to be interviewed. The interviews covered the following aspects: local language name of observed disease and other common diseases in their area, date of onset of observed disease, number of diseased and died animals. Others were, clinical/post mortem signs, disease history in their area, nearby affected house hold, treatment done, vaccination history, contact flocks, new animals brought in one previous month and wildlife contacts and cases experienced in wildlife recently (File S2).

The field team visited the households and observed the flocks from a distance, followed by estimation of flock size and identifying sick animals. Thereafter, sick animals were picked for clinical examination. On clinical examination, the first thing was determining the age by using dentition and compare with owner information. Then rectal temperature was measured using a clinical thermometer, other clinical signs including ocular/nasal discharge, oral nasal lesions, respiratory signs and diarrhoea were recorded. For clinical cases with temperature above 38.5^{o}C or cases with ocular discharges, a lateral flow devise (LFD) for PPR rapid test diagnosis was used to test whether the case was PPR or not as described by Jones *et al.* (2020). In case of LFD positive in one animal in the herd, samples were collected for laboratory confirmation on the tested animal and other 1 to 5 animals in the flock. The collected samples included pair of swabs (ocular, nasal and oral), blood in plain vacutainer tubes for sera, whole blood in ethylene diamine tetra acetic acid (EDTA), faeces and ticks in case they were available.

The whole blood in EDTA was processed by adding lysis buffer and serum was separated from clotted blood. Samples were stored at −163°C in liquid nitrogen until the end of the fieldwork, after which they were transported to the Sokoine University of Agriculture (SUA) where they were stored at -80^{o}C. Faecal samples were shipped in dry ice to the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France, where they were stored at −80 °C.

Laboratory tests

All serum samples were analysed using c-ELISA test, while all swabs were analysed by RT- qPCR. Positive samples on RT-qPCR were analysed by conventional PCR to get PCR products that were sent to Pirbright Institute for sequencing.

Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-qPCR)

Ocular swab samples (n=160) were screened for the presence of PPR virus nucleic acid by real-time reverse transcription-polymerase chain reaction (TaqMan® T-qPCR), following the method described earlier (Batten *et al.*, 2011). All reactions were run with Nigeria 75/1 vaccine strain as a positive control and nuclease-free water as the negative control. Nucleic acid was extracted from 100 µL of the swab samples (nasal or ocular) and or PPRV rapid diagnostic test fluid using the KingFisher TM Flex extraction system (ThermoFisher Scientific, Paisely, UK), following the manufacturer's instructions. The primers used was the N gene.

Sequencing and phylogenetic analysis

RT-qPCR positive samples were selected for gel-based polymerase chain reaction (PCR) and obtained PCR products were sequenced, with the aim of determining the lineages of the PPRV from the samples. The viral RNA was reverse transcribed and the C-terminus of

the N gene was amplified. The partial N gene sequences were compared to the available sequences in the GenBank by Blasting. Four sequences generated in this study were used to construct a neighbourhood-joining phylogenetic tree.

Results

During this study, a total of 78 flocks were examined in Karatu (n=10), Longido (n=9), Meatu (n=7), Monduli (n=11), NCAA (n=13), Ngorongoro (n=18) and Serengeti (n=10). The flocks examined in Ngorongoro district (n=7) where PPR was confirmed, were from Loliondo and Sale divisions the PPR confirmation was done using qPCR test (Figure 2). Whereas only one flock from Karatu and one flock from NCAA as part of Ngorongo district were confirmed positive. LFD confirmed PPR in Longido (n=3), Ngorongoro (n=7) and Serengeti (n=1). Out of six study districts, PPR was confirmed in Karatu, Longido, Ngorongoro and Serengeti districts using LFD and qPCR tests (Table 1). In Ngorongoro district, PPR was confirmed in all three divisions in the following wards: Loliondo (Oloipiri, Arash, Soitsambu and Enguserosambu), NCAA (Olbalbal) and Sale (Samunge and Malambo). In Karatu district, PPR was confirmed in Mang'ola, the ward bordering with NCAA. In Longido the disease was confirmed in Sinya and Engarenaibor wards, the wards found at the border with Kenya. Whereas, in Serengeti the disease was confirmed in Kyambahi ward.

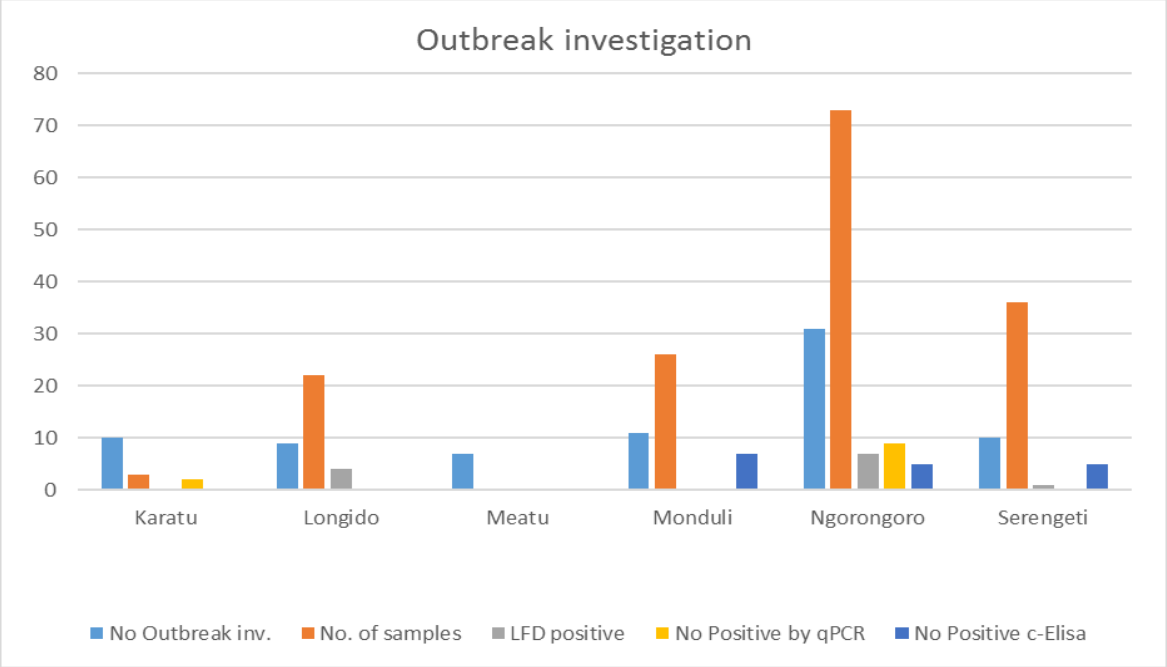


Figure 2: Study districts, outbreak investigation, samples per district, LFD and qPCR positive results

Table 1: Districts, wards and villages with PPR confirmed cases by lateral flow devise and qPCR

District	Ward	Village
Karatu	Mang'ola	Malekchandi
Longido	Sinya	Ildonyoo
	Engarenaibor	Ngoswak
Ngorongoro	Arash	Mbukeni
	Enguserosambu	Naani
	Esere	Laitore
	Soitsambu	Kritalo
	Oloipiri	Lopor
	Enduleni	Enduleni
	Nainokanoka	Ilkepus
	Misigiyo	Longojoo
	Olbalbal	Irkipori
	Samunge	Samunge
	Malambo	Sanjani
Serengeti	Nyichoka	Kyambahi

About 60% of animals confirmed to be PPR positive were aged below six months, although some confirmed PPR positive animals were aged above three years. Mucoid nasal discharge was observed in 55% of the cases. In addition, about 55% of cases had

oral-nasal lesions. About 27% of confirmed cases experienced coughing and 18% had difficulty breathing, while about 55% of cases did not have any respiratory signs. Diarrhoea was manifested only to 45.5% of the confirmed cases, with only one animal found to have bloody diarrhoea. About 45.5% of the PPR confirmed cases did not show any kind of diarrhoea, with one animal having perianal epithelial tissue eruption. Of the confirmed cases by qPCR, only 27% tested positive by the lateral flow devise used for field confirmation of cases. The selection criteria for clinical examination were animals with temperature above 40^{o}C and lacrimation. The confirmed cases had temperature ranging from 38.3^{o}C to 41.3^{o}C, about 70% had lacrimation, 20% did not have ocular discharges and one animal had mucopurulent ocular discharge.

Table 2: The proportional chi-squire test at 95% CI and 1 degree of freedom for different attributes on the confirmed cases of PPR in Mara Serengeti area

S/n	Category	Chi squire Value	P Value
1	Species (Sheep and Goat)	7.840	0.0051
2	Sex (Male and Female)	1.00	0.3173
3	Age (≥6 month and < 6 month)	7.840	0.0051
4	Body temperature (≥38.5 and < 38.5)	67.240	< 0.0001
5	Ocular signs (conjunctivitis, lacrimation and purulent discharge) - Yes/No	40.960	<0.0001
6	Nasal discharge(Mucopurulent/purulent/watery) -Yes/No	1.00	0.3173
7	Oral-nasal lesions (gum congestion, necrotic tissues on gum/lips/tongue, ulceration of lips/mouth) - Yes/No	1.00	0.3173
8	Respiratory signs (coughing, difficult breathing and rapid respiration) - Yes/No	1.00	0.3173
9	Diarrhoea (watery, blood and soil hind quarter) -Yes/No	1.00	0.3173

RT-PCR

Figure 3: PCR products visualized under UV Trans illuminator showing nucleoprotein gene amplicons, T2, T3, T10, T11 and T13 PPRV from Goat and sheep

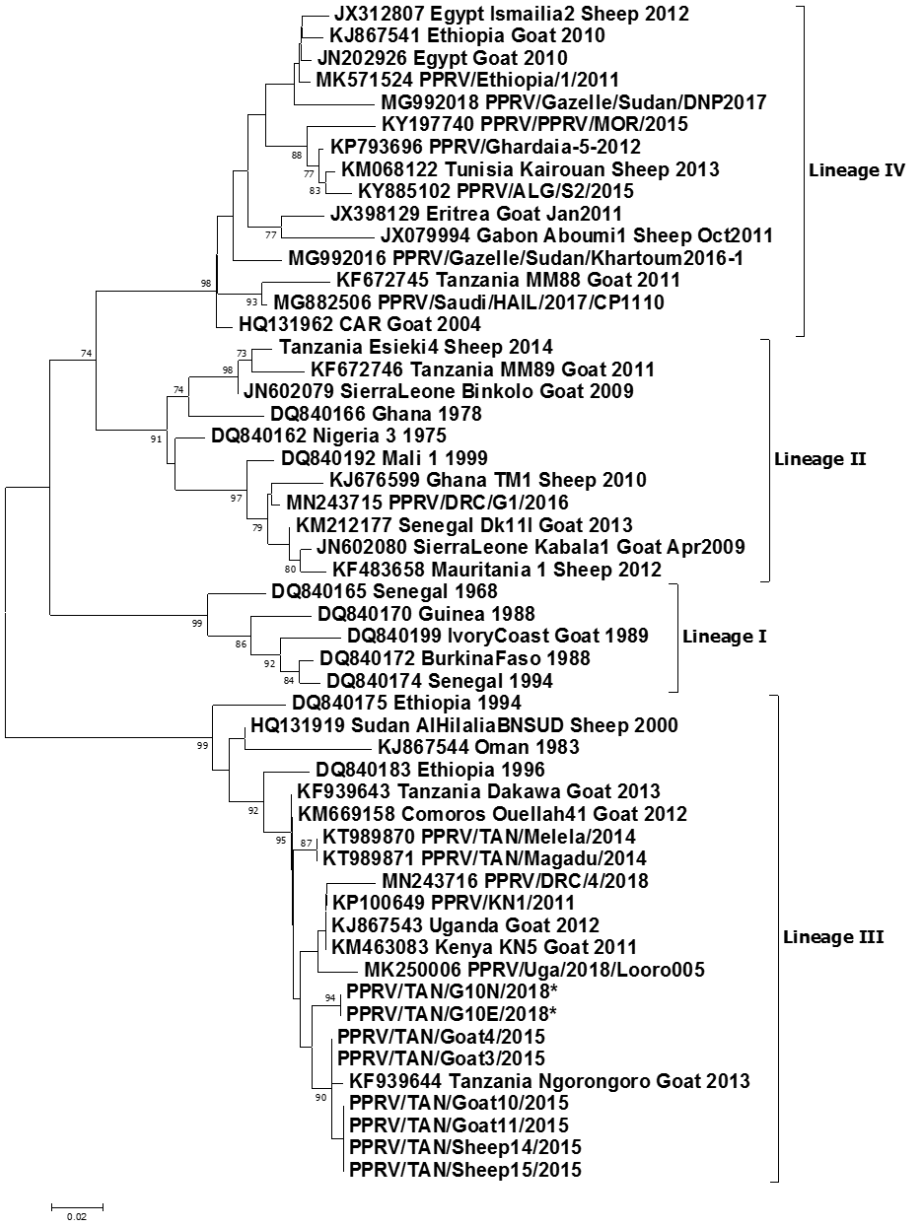


Figure 4: Phylogenetic tree of PPR viruses based on the N gene constructed using the neighbour-joining method in MEGA 7 software based PPRV partial N-gene sequence

Figure 5: A: ocular and nasal discharge, B: rough hair coat and shivering, C: perianal epitherial tissue eruption and D: confirmed case of PPR using rapid test kit

Interviews

From semi structured interviews, it was realised that pastoral communities were much aware of animal syndromes and have traditional names and remedies unlike the agro-pastoral communities who mostly use Swahili terms. However, it was noticed that all ethnic groups had no specific name for PPR, rather there were a number of syndromes associated with presence of PPR or PPR-like diseases.

Table 3: Common syndromes associated with PPR and other PPR-like diseases

Syndrome	Ethnic group traditional name				
	Balbaig	**Maasai**	**Irak**	**Kurya**	**Sukuma**
Lung disease/ respiratory	Dagshaigiti	Orkipei	Khumpaa	Ekihaha	Mabupu
Diarrhoea	Gheshaideda	Ngorotik	Deshimoo		
Oral lesion/orf	Daktuta/		Drihii	Amasisa	Lhumeme
Nasal discharge					
Ocular discharge					
Enlarged bile		Ordwaa			
Nervous signs	Gdihenga	Ormilo	Parampili	Ekisengoro	Lusalo
Pox lesions		Ormoloji	Quluhay	Amasondo	Ndubi
Fever	Selepta				

Interviewed individuals in the study area reported Sheep and goats to be important assets for livelihoods, they are source of food and income. They reported pastoral production system as the predominant system, characterised by extensive mobility in search for pasture and water especially during drought period. Movement of animals from infected area to clean area on disease avoidance was a common practise. Often times, animals are either sold or purchased from livestock keepers, traders or brokers in live animal markets, primary or secondary, premises located at nearby villages, districts, regions for breeding, fattening, trading or slaughter. To the markets and from the markets animals are moved on foot. On grazing areas meeting herds from distant villages and even districts and wildlife is a common practise although wild animals mixing with livestock is not a common feature.

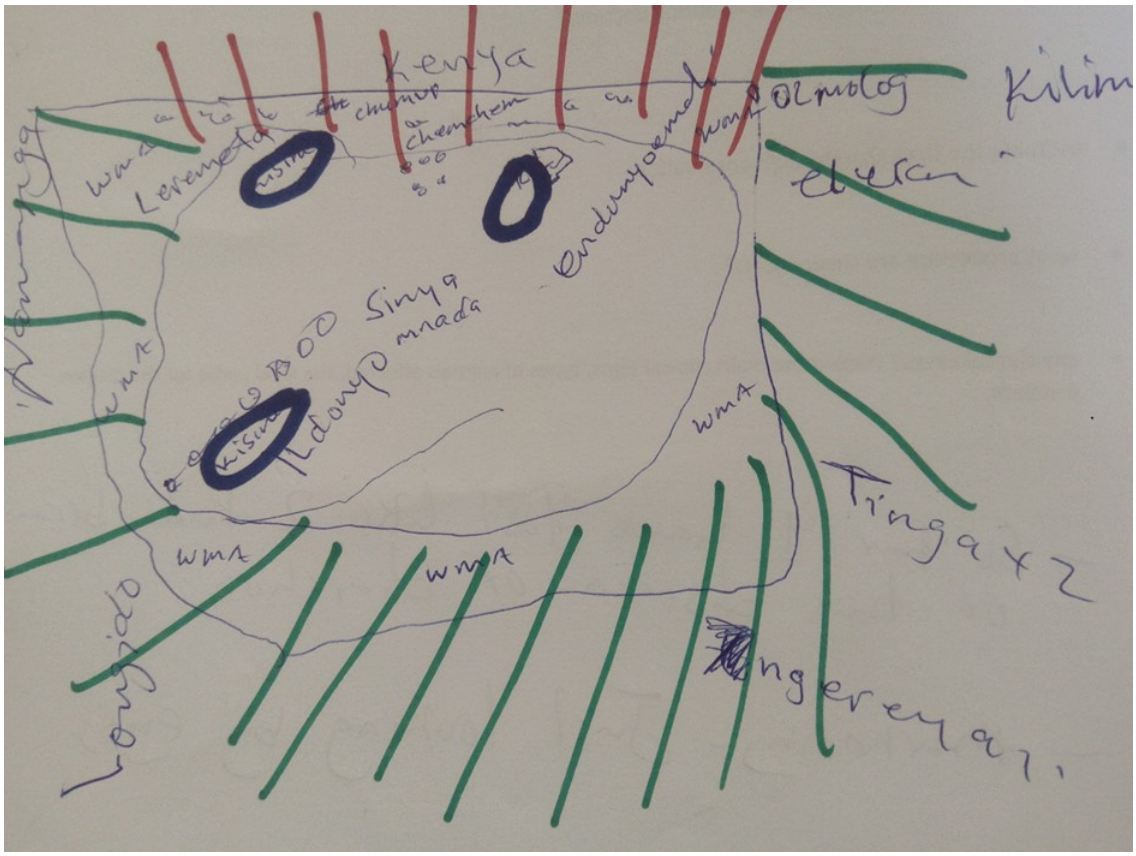


Figure 6: Participatory map of Sinya Ward in Longido district; Green shadow is Wildlife Management Area and grazing area, red shadow is a salt leaking area and blue circles are water wells for animals drinking

Discussion

In the present study clinical cases of PPR reported by livestock field officers and livestock keepers from different localities in the study area were confirmed by rapid field test (LFD) and RT –qPCR. Serological analysis done by c-ELISA test was used to determine levels of sero-conversion in the study area in localities where there was history of PPR clinical signs and where PPR was confirmed by other tests. The clinical signs observed during clinical examination and reported by livestock keepers during the study period were similar to what has been reported in other studies (Muse *et al.*, 2012; Kgotlele *et al.*, 2014; Jones *et al.*, 2020).

However, in this study most cases presented in a milder form especially those confirmed in Karatu district. Among the PPR confirmed animals, animals which had nasal discharges, oral-nasal lesions, respiratory signs and diarrhoea, the most common PPR clinical signs were many than those which did not show those signs. There was no statistically significant difference in proportions of animals presenting with the most common PPR signs and those that did not show those signs (Table 2). Moreover, perianal epithelial tissue eruption was observed in one of the clinical cases that later was confirmed to be a PPR case. The animal had no any sign of diarrhoea but was found with other clinical signs which included nasal discharge and lacrimation, an observation that has never been reported in other studies. Ocular signs, increased body temperature (>38.5ºC) and age of affected animals (≥6month) were found to be critical features in clinically diagnosed and confirmed PPR cases in endemic settings like Serengeti ecosystems. Moreover, in mixed species production, goats seemed to be more affected compared to sheep. Comparing the proportion of confirmed cases presenting with ocular signs, increased body temperature and species to those without these features. The features can be considered in the standard case definition when diagnosing PPR suspected cases based on clinical signs in endemic areas. Moreover, these features can be used in differentiating PPR from other diseases with PPR like signs.

In our study area, specifically in Ngorongoro, the population of sheep was higher than that of goats, yet sheep were fewer during clinical examination compared to goats. Based on RT-qPCR results, goats were mostly affected compared to sheep in the study area. The PPR clinical signs in sheep were less severe when compared to those in goats. However, it is not clear whether this was due to the small number of diseased and examined sheep at the time of the outbreak or was due to the virulence of the circulating lineage of PPRV. In this study, the confirmed PPR cases found to be more severe in young animals aged less

than six months. This observation is in line with other studies where in PPR endemic areas adult animals normally will have already developed immunity from past infection and young animals over four months and less than six months are highly susceptible because that period coincides with the time when maternal immunity tend to decline (Awa *et al.,* 2002; Sanne *et al.,* 2006; Balamurugan *et al.,* 2012). Moreover, there was no statistically significant difference between sex, a finding which is described in the literature (Jones *et al.,* 2020).

The sequences from confirmed PPRV cases were used to construct a phylogenetic tree. It was found that the PPRV cases from all sampled points belonged to lineage III. Previous studies conducted in the same area at different periods found lineages II and III circulating in the area (Kgotlele *et al.,* 2014; Kivaria *et al.,* 2014; Jones *et al.,* 2020). Therefore, the study finding is similar with the previous studies on the area, suggesting identical virus circulates from the first outbreak reported in 2008 in Ngorongoro.

Traditionally, PPR is well known among pastoralists compared to agro-pastoralists residing in areas around the Serengeti Ecosystem. During interviews, pastoralists named PPR and other PPR-like diseases based on the presenting syndrome. However, not much was found to be known among agro-pastoralists. This could probably be because livestock keeping is not a primary means of livelihood among agro-pastoralists, who depend on both crop production and livestock keeping for their livelihood. According to the interviewed pastoralists it was revealed that although PPR seems to be a problem, it is not a major problem if compared with clinical cases associated with nervous signs. According to them the clinical cases associated with nervous signs are not curable while cases associated with clinical signs similar to that of PPR can be prevented through vaccination and animals respond well to treatment unlike cases of nervous signs. There was no known

traditional remedy for PPR among both the pastoralist and agro-pastoralist communities, indicating that there was no accumulated traditional knowledge about PPR, suggesting that the disease has not been in the area for a long period. This is in line with other studies which reported that PPR was officially confirmed in Tanzania in year 2008.

Pastoral production system is reported to be the most common with agro-pastoral production system in the area associated with long distance movement of livestock. Sheep and goats are considered as an important resource for livelihood as a source of food and income. PPR has been confirmed in this area with multiple land use arrangements, where livestock coexist with wildlife sharing grazing land, watering points, and salt leaking areas, thereby coming into close contact (Figure 1). Considering the mode of PPR transmission, there is a high chance of PPR spilling over to wildlife sharing grazing pasture, water resources and mineral leak with livestock. The possibility of clinical PPR occurrence/outbreak in wildlife in the Serengeti ecosystem is inevitable. An indication of spill over of PPR virus from livestock to wildlife has already been reported in previous studies by detecting PPRV antibodies in a number of wildlife species (Mahapatra *et al.*, 2015). As well PPR has been confirmed in cross border villages (Ildonyoo and Naan) where livestock keepers from Kenya and Tanzania share grazing resources and salt leak areas (Figure 1 and Figure 3). This situation increases chances of disease moving between the two countries. Managing a TAD like PPR in such a situation needs cross border agreements on surveillance and control.

Conclusion and Recommendations

PPR cases were confirmed in the study districts, including all divisions of Ngorongoro district, the epicentre of greater Serengeti Ecosystem. In Karatu and Longido districts the disease has been confirmed in a ward bordering the NCAA in Karatu and that borders with

Narok and Kajiado counties of Kenya in Longido. The confirmed cases were higher among young sheep and goats coexisting with wildlife. The disease presented itself with mild clinical signs unlike in a naïve population where normally the disease is severe and occurs in form of outbreaks. Lineage III was found to be prevailing in the area. The pastoralists are aware of the disease clinical signs, even though in some cases the disease was confused with other diseases with similar clinical manifestation. Co-existence of wildlife and livestock in the area where PPR has been confirmed increases risk of PPR spilling over to wildlife. Nevertheless, confirmation of PPR in the border wards with Kenya necessitates collaboration of conservation authorities and countries on PPR surveillance and Control by vaccination in order to prevent the possibilities of disease spread and maintenance among endangered wild small ruminants species and neighbouring country.

Acknowledgements

The authors would like to acknowledge the Royal Veterinary College - London, Sokoine University of Agriculture, local government authorities, Directorate of Veterinary services - Tanzania, Tanzania Wildlife Research Institute, Pirbright Institute and Southern Africa Centre for Infectious Diseases Surveillance for the roles they played in making this work possible. The authors are also grateful to the Global Challenges Research Fund of the UK government through the Biotechnology and Biological Sciences Research Council for providing support for the research titled, Pathway to Peste des Petits Ruminants Virus Elimination - methods for complex ecosystems in a mixed wildlife livestock ecosystem in East Africa BB/P023002/1. The publication of the paper supported by the PPR translational award GRTA (EP/T015381/1) to ensure open source for all colleagues.

References

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

Abubakar, M. and Munir, M. (2014). Peste des Petits Ruminants virus: An emerging threat to goat farming in Pakistan. *Transboundary and Emerging Diseases*, 61(SUPPL1): 7 - 10.

Anderson, J. and McKay, J. A. (1994). The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implications to rinderpest control programmes. *Epidemiology and Infection* 112(1): 225 -231.

Awa, D. N., Ngagnou, A., Tefiang, E., Yaya, D. and Njoya, A. (2002). Post vaccination and colostral peste des petits ruminants antibody dynamics in research flocks of Kirdi goats and Foulbe sheep of North Cameroon. *Preventive Veterinary Medicine* 55(4): 265 - 271.

Aziz-ul-Rahman, W. J. J., Abubakar, M., Shabbir, M. Z. and Rossiter, P. (2018). Peste des petits ruminants in wild ungulates. *Tropical Animal Health and Production* 50(8): 1815 - 1819.

Balamurugan, V., Sen, A., Venkatesan, G., Rajak, K. K., Bhanuprakash, V. and Singh, R. K. (2012). Study on passive immunity: Time of vaccination in kids born to goats vaccinated against peste des petits ruminants. *Virologica Sinica*

27(4): 228 - 233.

Baron, M. D., Diop, B., Njeumi, F., Willett, B. J. and Bailey, D. (2017). Future research to underpin successful peste des petits ruminants virus (PPRV) eradication. *Journal of General Virology* 98(11): 26 35 - 2644.

Batten, C. A., Banyard, A. C., King, D. P., Henstock, M. R., Edwards, L., Sanders, A. and Barrett, T. (2011). A real time RT - PCR assay for the specific detection of Peste des petits ruminants virus. *Journal of Virological Methods* 171(2): 401 - 404.

Bodjo, S.C., Couay-Hymann, E.K. M. and Therese, D. T. (2006). Assessment of the duration of maternal antibodies specific to the homologous. *Biokemistri* 18(2): 99–103.

Diallo, A., Minet, C., Le Goff, C., Berhe, G., Albina, E., Libeau, G. and Barrett, T. (2007). The threat of peste des petits ruminants: progress in vaccine development for disease control. *Vaccine* 2005(30): 5591 - 5597.

Jones, B. A., Mahapatra, M., Chubwa, C., Clarke, B., Batten, C., Hicks, H. and Parida, S. (2020). Characterisation of peste des petits ruminants disease in pastoralist flocks in Ngorongoro district of northern Tanzania and bluetongue virus co-infection. *Viruses* 12(4): 1 - 14.

Jones, B. A., Muhammed, A., Ali, E. T., Homewood, K. M. and Pfeiffer, D. U. (2020). Pastoralist knowledge of sheep and goat disease and implications for peste des petits ruminants virus control in the Afar Region of Ethiopia. *Preventive*

Veterinary Medicine 174(10): 28 - 42.

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

Kivaria, F.M., Kwiatek, O., Kapaga, A. M., Swai, E. S., Libeau, G., Moshy, W. and Gladson, J. (2014). The incursion, persistence and spread of peste des petits ruminants in Tanzania: Epidemiological patterns and predictions. *Onderstepoort. Journal of Veterinary Research* 81(1): 1 - 10.

Kock, R. A., Orynbayev, M. B., Sultankulova, K. T., Strochkov, V. M., Omarova, Z. D., Shalgynbayev, E. K. and Parida, S. (2015). Detection and Genetic Characterization of Lineage iv Peste Des Petits Ruminant Virus in Kazakhstan. *Transboundary and Emerging Diseases* 62(5): 470 - 479.

Kumar, N., Chaubey, K. K., Chaudhary, K., Singh, S. V., Sharma, D. K., Gupta, V. K., and Sharma, S. (2013). Isolation, identification and characterization of a Peste des Petits Ruminants virus from an outbreak in Nanakpur, India. *Journal of Virological Methods* 189(2): 388 – 392.

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

Mahapatra, M., Sayalel, K., Muniraju, M., Eblate, E., Fyumagwa, R., Shilinde, L. and Kock, R. (2015). Spillover of Peste des Petits Ruminants Virus from Domestic to Wild Ruminants in the Serengeti Ecosystem, Tanzania. *Emerging Infectious*

Diseases 21(12): 2230–2234.

Mdetele, D. P. and Kassanga, C. (2014). Seroprevalence of foot and mouth disease in Tanzania. *International Journal of Infectious Diseases* 21: 451 – 452.

Munir, M., Zohari, S., Saeed, A., Khan, Q. M., Abubakar, M., Leblanc, N. and Berg, M. (2012). Detection and Phylogenetic Analysis of Peste des Petits Ruminants Virus Isolated from Outbreaks in Punjab, Pakistan. *Transboundary and Emerging Diseases* 59: 85 - 93.

Munir, M. (2014). Role of wild small ruminants in the epidemiology of peste des petits ruminants. *Transboundary and Emerging Diseases* 61(5): 411 - 424.

Muse, E. A., Karimuribo, E. D., Gitao, G. C., Misinzo, G., Mellau, L. S. B., Msoffe, P. L. M. and Albano, M. O. (2012). Epidemiological investigation into the introduction and factors for spread of Peste des Petits Ruminants, southern Tanzania. *Onderstepoort Journal of Veterinary Research* 12(2): 1 - 6.

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

Rahman, M. Z., Haider, N., Gurley, E. S., Ahmed, S., Osmani, M. G., Hossain, M. B. and Rahman, M. (2018). Epidemiology and genetic characterization of Peste des petits ruminants virus in Bangladesh. *Veterinary Medicine and Science* 4(3): 1 - 11.

Schulz, C., Fast, C., Schlottau, K., Hoffmann, B. and Beer, M. (2018). Neglected Hosts of Small Ruminant Morbillivirus. *Emerging Infectious Diseases* 24(12): 2334 -

2337.

Sen, A., Saravanan, P., Balamurugan, V., Bhanuprakash, V., Venkatesan, G., Sarkar, J. and Singh, R. K. (2014). Detection of subclinical peste des petits ruminants virus infection in experimental cattle. *Virus Disease* 25(3): 408 - 411.

Sharma, K. K., Kshirsagar, D. P., Kalyani, I. H., Patel, D. R., Vihol, P. D. and Patel, J. M. (2015). Diagnosis of peste des petits ruminants infection in small ruminants through in-house developed indirect ELISA: Practical considerations. *Veterinary World* 15: 443 - 448.

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

Swai, W., Moshy, E. and Mbise, J. K. (2011). Serological evidence of camels exposure to peste des petits ruminants virus in Tanzania. *Research Opinion in Animal and Veterinary Science* 1(5): 325–329.

Taylor, W. P. and Barrett, T. (1946). *Diseases of sheep. Veterinary medicine* 41: 53 - 55.

Travis, D. A., Watson, R. P. and Tauer, A. (2011). The spread of pathogens through trade in wildlife. *OIE Revue Scientifique et Technique* 30(1): 219 – 239.

Truong, T., Boshra, H., Embury-Hyatt, C., Nfon, C., Gerdts, V., Tikoo, S. and Babiuk, S. (2014). Peste des petits ruminants virus tissue tropism and pathogenesis in

sheep and goats following experimental infection. *PLoS ONE* 9(1): 23 – 56.

Published paper IV

Modified netting technique for capturing gazelles in Serengeti, Ngorongoro and Loliondo, Tanzania

[1]Mdetele. D[*], [1]Misinzo, G. [1]Komba, E. [3]Shemanche, J. [3]Eblate, E. [3]Mdaki, M. [3]Keyyu, J. and [2]Kock, R.

[1]Sokoine University of Agriculture, College of Veterinary Medicine and Biomedical sciences P.O. Box 3015, Chuo Kikuu Morogoro.

[2]Royal Veterinary College, Hawkshead Lane, Hatfield, Herts AL9 7TA, UK

[3]Tanzania Wildlife Research Institute, P.O Box 661, Arusha, Tanzania

Received: 9 June 2020 | Revised: 20 August 2020 | Accepted: 16 September 2020

DOI: 10.1111/aje.12813

ARTICLE

African Journal of Ecology WILEY

Modified netting technique for capturing gazelles in Serengeti, Ngorongoro and Loliondo, Tanzania

Daniel Mdetele[1] | Gerald Misinzo[1] | Erick Komba[1] | Justin Shemanche[2] | Ernest Eblate[2] | Maulid Mdaki[2] | Julius Keyyu[2] | Richard Kock[3]

[1]College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Chuo Kikuu, Morogoro, Tanzania

[2]Tanzania Wildlife Research Institute, Arusha, Tanzania

[3]Royal Veterinary College, Hatfield, UK

Correspondence
Daniel Mdetele, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3015, Chuo Kikuu, Morogoro, Tanzania.
Email: daniel.mdetele@sacids.org

Funding information
Global challenges research fund (GCRF), Grant/Award Number: BB/P023002/1

Abstract

During serological surveillance of peste des petits ruminants (PPR) disease, it required capture of randomly selected herds of gazelles as part of a study to determine the epidemiological role of these species in the circulation of peste des petits ruminants virus (PPRV). The study targeted capturing 135 Grant's gazelles (*Gazella granti*) from the Serengeti ecosystem, Tanzania. A modified netting technique was used aiming at providing safe, efficient and cost-effective method for capture of gazelles. Locally available materials were used, and wildlife professionals guided the process of manufacturing supporting frame for the nets. Twenty (20) black metal pipes, 20 metal bars, four nets and three vehicles were used in the procedure. A total of 136 Grant's gazelles and nine Thomson's gazelles were captured in three missions. The Grant's gazelles were captured as per sample size calculated in all locations: Loliondo (n = 25), Serengeti National Park (n = 44) and Ngorongoro Conservation Area (NCA) (n = 67) using less time and minimum cost than estimated. Injuries of three fawns (2%) inadvertently captured with the groups of adults and sub-adult animals were recorded. Comparing with 2014 and other studies, modified netting technique showed high animal and operator safety levels with minimal injuries. With this technique, it was possible to capture even flighty animals that behave nervously because of hunting and other human activities, including Thomson's gazelles, a species previously found to be difficult to capture by netting.Keywords

capture, disease surveillance, gazelles, netting, Tanzania

Résumé

Lors de la surveillance sérologique de la peste des petits ruminants (PPR), il a fallu capturer des troupeaux de gazelles sélectionnés au hasard dans le cadre d'une étude visant à déterminer le rôle épidémiologique de ces espèces dans la circulation du virus de la peste des petits ruminants (VPPR). L'étude visait à capturer 135 gazelles de Grant (*Gazella granti*) présentes dans l'écosystème du parc national du Serengeti, en Tanzanie. Une technique modifiée de capture par filets a été utilisée dans le but d'assurer une méthode sûre, efficace et économique pour la capture des gazelles. Des matériaux disponibles localement ont été utilisés et des experts de la faune ont guidé le processus de fabrication du cadre porteur des filets. Vingt (20) tuyaux en métal noir, 20 barres métalliques, quatre filets et trois véhicules ont été utilisés dans

le cadre de la procédure. Au total, 136 gazelles de Grant et neuf gazelles de Thomson ont été capturées au cours de trois missions. Les gazelles de Grant ont été capturées selon la taille de l'échantillon calculée sur tous sites; Loliondo (n = 25), parc national du Serengeti (n = 44) et la zone de conservation du Ngorongoro (NCA) (n = 67) en moins de temps et pour un coût minimum moins élevé que prévu. Trois faons (2%) capturés par inadvertance avec les groupes d'animaux adultes et subadultes ont été blessés. En comparaison avec l'année 2014 et d'autres études, la technique modifiée de capture par filet a démontré des niveaux élevés de sécurité pour les animaux et les opérateurs avec un nombre minimal de blessures enregistré. Grâce à cette technique, il a même été possible de capturer des animaux instables qui se comportent nerveusement en raison de la chasse et d'autres activités humaines, notamment des gazelles de Thomson, une espèce auparavant difficile à capturer au filet.

1 | INTRODUCTION

The capture of free-ranging wildlife has always been a difficult but necessary part of population management, animal monitoring through marking or radio collaring for remote sensing, disease investigation, relocation and many other conservation practices (Gehr, 2010; Webb et al., 1996). There has been advancement in capture and handling methods to minimise the amount of stress imposed on animals and to reduce the risk of mortality at the time of capture. It is extremely important that the best practices are known, published and used for ethical and welfare reasons (Mmmalogists, 1998). This will assist in achieving objectives in an efficient, cost-effective manner and minimise mortality in the management of rare, threatened or endangered species of wildlife. Methods used for physical capture are reported elsewhere (Farst & Fowler, 2010; Ferreira, 2016; Gehr, 2010; Lekolool, 2012; Locke et al., 2004; Denicola et al., 2000; Webb et al., 1996) , reviewed in texts (Laubscher et al., 2015) and developed into training and field manuals (Kock et al., 2012) .

There are relatively few reports of netting gazelles in East Africa with exception of Kenya Wildlife Services (KWS) internal reports (KWS 1996; R. Kock, personal communication, 2019). In Kenya, in the 1990s, on the plains between Longonot and Suswa Volcanoes in the Rift Valley, over one hundred Thomson's gazelles (*Eudorcas thomsonii*) were caught through a simple drive chasing using over 200 m of extended short cotton nets. These were captured successfully for translocation to the Middle East (KWS 1996; Kock, personal communication 2019). In Ngorongoro district of Tanzania, in 2014 and 2015, 27 Grant's gazelles (*Nanger granti*) and one Thomson's gazelle (*Eudorcas thomsonii*) were netted in an earlier phase of peste des petits ruminants (PPR) research in the region. The research aimed at investigating the spillover of the PPR virus from goats and sheep co-existing with these species through serological surveillance. These animals were netted by using a combination of a net boma (fixed perimeter with gum poles) and internal drop nets (Cape Netting PLC RSA nylon cotton rope net 30 m × 3 m × 150 mm mesh × 4 mm Tex Pes Br. and 50 m × 3 m × 150 mm square mesh × 5 mm Tex Pes Br.). This technique was used successfully with no injuries or mortalities (Mahapatra et al., 2015; Parida, 2017). One constraint of this method was the time and effort in setting up the system which usually resulted in only one attempt at capture per day. The initial research was extended to include a much larger sample size across a larger landscape in order to achieve key objectives of the PPR research for improved understanding of the PPR virus epidemiology. This required capture of randomly selected herds of gazelles as part of a study to determine the epidemiological role of these species in the circulation of peste des petits ruminants virus (PPRV). The number of animals to be captured was calculated on a statistical basis to ensure the minimum interventions to achieve significance in the analysis. This is essential data for ensuring the eradication strategy of PPR in the region takes into account the role of wildlife in persistence of the disease. Therefore, a budget for chemical immobilisation and netting were prepared by Tanzania Wildlife research institute (TAWIRI). The two methods were compared on different aspects. However, netting was again applied in 2019 with modifications. Previous experience in the region and reference to literature were used as a basis for designing a capture technique for disease surveillance in the Serengeti ecosystem over the period 2014–2019. One hundred and thirty-six (136) Grant's gazelles were captured from 27 sites in the Greater Serengeti ecosystem, inside and outside of wildlife protected areas. This also included opportunistic capture of nine Thomson's gazelles, a notoriously difficult species to capture using mobile net systems. A modified netting method used for capture of these species of gazelles, for interventions and health surveys is reported here. Although large-scale capture of blesbok (*Damaliscus pygargus phillipsi*), springbok (*Antidorcas marsupialis*), oribi (*Ourebia ourebi*), impala (*Aepyceros melampus*) and tsessebe (*Damaliscus lunatus*) has been reported (Laubscher et al., 2015) and their associated mortalities documented (Hofmeyr et al., 1976), the scale of safe capture of Grant's gazelle (*Nanger granti*) undertaken is unprecedented, to the author's knowledge.

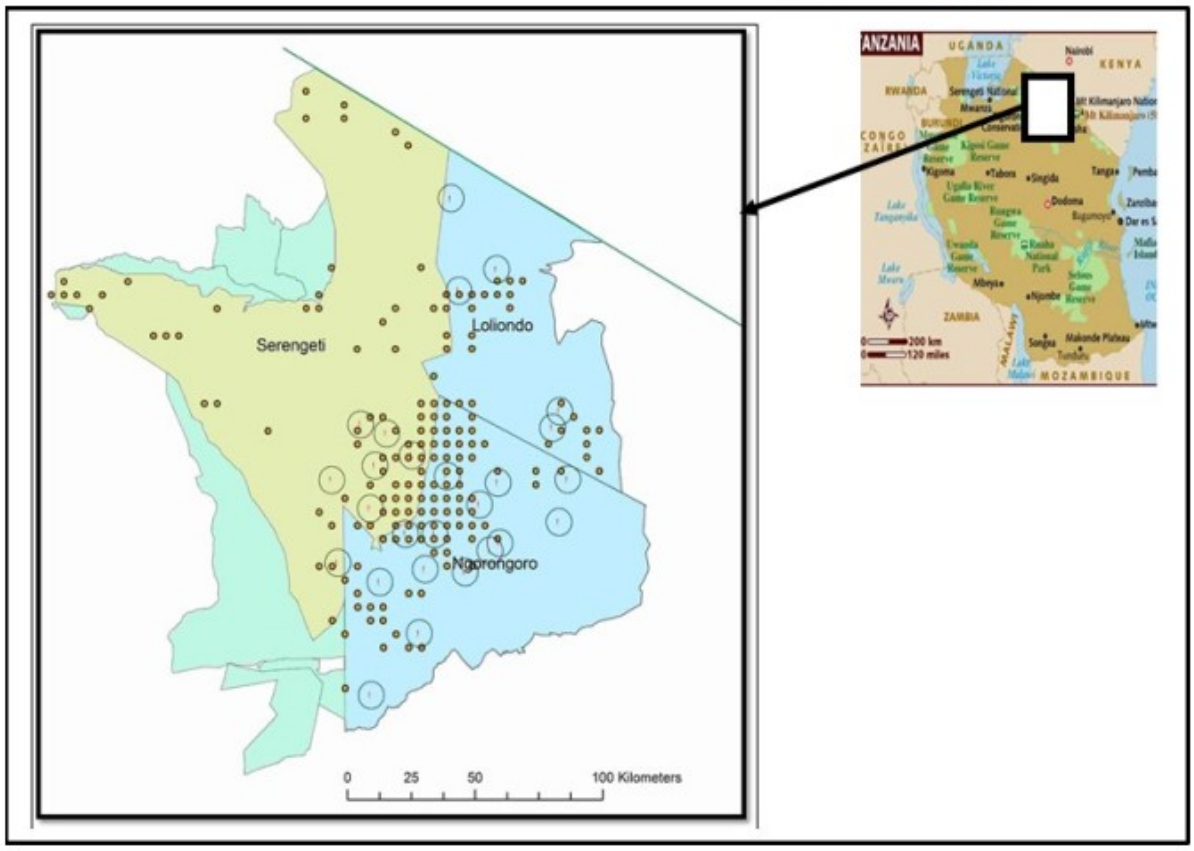


FIGURE 1 Map showing greater Serengeti Ecosystem and distribution of Grant's gazelles (*Nanger granti*) (small circles) in Serengeti, Ngorongoro and Loliondo, and locations where sampled Grant's were captured (larger circles)

2 | MATERIALS AND METHODS

2.1 | Study area

The study was carried out in the greater Serengeti ecosystem which comprises of Serengeti National Park (SNP), Ngorongoro Conservation Area Authority (NCAA) and Loliondo Game controlled Area (LGCA) (Figure 1). These areas are in Ngorongoro district, Arusha region and Serengeti district in Mara region; all found in northern Tanzania. A greater part of this area is a conserved area with areas strictly for wildlife (SNP), and others with multiple land use where wildlife co-exist with livestock (NCAA and LGCA).

2.2 | Materials

- Black pipes—ordinary round metal pipes used in households for gas lines and other appliances in Tanzania (22 mm diameter).
- Furniture pipes—softer metal pipes available in Tanzania (22 mm diameter)
- Iron bars 70 m (22 m diameter)—round metals used in construction, pointed on one end for easy ground pinning by hammer.
- Animal capture nets
- Hammer for ground pinning of the iron bars
- Three vehicles (two station wagons and one pick up)

2.3 | Modified netting method

The framework for the nets was manufactured by local Tanzanian technicians using locally available materials. Materials used included (a) 20 pieces (pcs) of metal pipes measuring 3 metre (m) tall and

FIGURE 2 Picture showing netting structure with pipes inserted into ground pinned metal bars and hanging nets

FIGURE 3 Picture showing vehicles herding gazelles towards the netting system

22 mm in diameter. The pipes had hooks welded 8 cm from both ends holding nets which allow rapid dropping of the net upon impact on the net by running animals chased by vehicles. (b) 20 pcs of iron bars measuring 70 cm long, 22 mm in diameter with one sharp end, ground pinned half way using a hammer at an interval of 10 n . The hooked metal pipes were inserted into the other half of ground pinned metal bars. (c) 50 m long and 3 m wide nets (Cape Netting PLC RSA nylon cotton rope net measuring 50 m × 3 m × 150 mm square mesh × 5 mm Tex Pes Br) hanged on the metal pipes hooks. The pipes were inserted into the iron bars to make a half-mooned shape-netting trap (Figure 2). Usually, four nets were used and their placement was designed to make escape difficult once the gazelles were herded to within 100 m of the net configuration. The nets were sited in areas where vehicles could move freely without excessive bush or ground obstacles. Once a group of gazelles was identified, three vehicles positioned in a V shaped manner gently pushed them towards the netting site from a distance of between 150 and 200 m (Figure 3).

On average the netting system had a diameter of 100 m. At the beginning of this work, furniture pipes were used exclusively; however later, these were changed because it was found that the furniture pipes were bending when bigger male Grant's gazelles bounced into the net. Thereafter, black pipes were used alternately with furniture pipes. Each of the two station wagons used had five personnel at a time, whereas the pickup had two personnel. After completion of activities, the pickup was also used to carry the netting system from one point to the other. During this work, most of the time was spent for travelling and logistics. On arrival at a sampling point, communications were made with the resident wildlife protection authority. They provided one or two officers to accompany the team to ensure that safety and welfare of animals were observed on each sampling site.

During each capture round, small groups of Grant's gazelles, 6–20 in number, were approached by the three vehicles, with the first vehicle in line with one end of the net system, the second vehicle on the other end and the third vehicle positioned in the middle and slightly behind the other two, displaying a shape resembling the horns of a buffalo (Figure 4). For the first 3–5 min, animals were pushed slowly at a speed between 30 and 40 kilometres (km) per hour (hr) towards the centre of the net system. When about 100 to 150 m from the entrance to the netting system the coordinating vehicle signalled to increase the speed up to 120–140 km/hr driving the gazelles into the net. This high speed was possible because the setting of nets considered areas with good terrain where a car could drive smoothly. This manoeuvre distracts the animals from the presence of the net and in a panic, they usually enter and hit the net, becoming safely entangled. The vehicles immediately stopped once the animals hit the nets and the capture team left the vehicles and safely restrained the entangled animals, whilst ensuring that they could breathe effectively and had no rope restrictions that could cause injury to the head, neck, legs or body. Thereafter, sampling equipment was brought and sample collection conducted. Sampling started with larger animals as it is much more difficult to restrain them for a prolonged time. Restraining of aggressive animals involved more than one person. Normal tranquilisers could be administered (Hofmeyr et al., 1976), but in this case, these were not used as the protocol was for immediate release after sampling and this

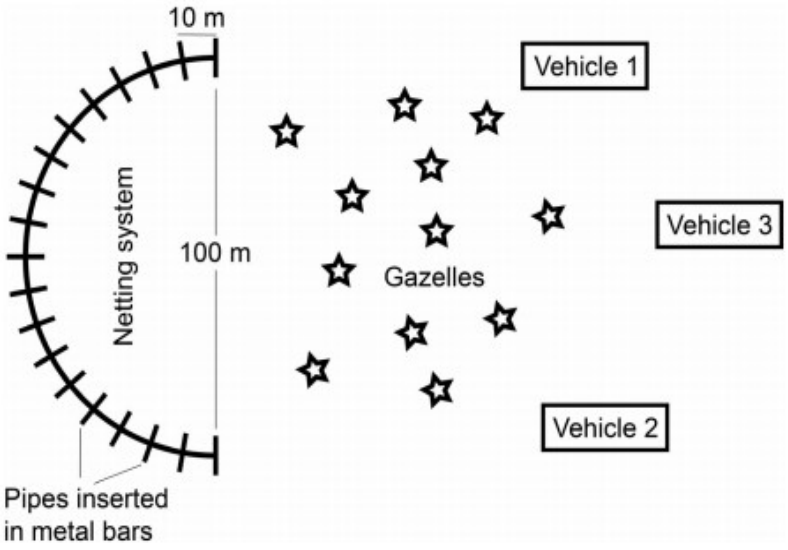


FIGURE 4 Picture showing netting system, vehicles and Grant's gazelles positioning during capture

TABLE 1 Location, age and sex of Grant's and Thomson's gazelles captured and sampled

	Grant's and Thomson's gazelles captured, age category and sex								
	Aged		Adult		Sub-adult		Young		
Location	Male	Female	Male	Female	Male	Female	Male	Female	Total
Loliondo	0		2	13	5	4	0	2	26
NCAA	0		23	30	6	8	5	3	75
Serengeti NP	0	2	18	20	1	3	0	0	44
Total	2		106		27		10		145

avoids any higher risk of predation from longer term effects of tranquilisers. When the animal was stable and secure, the biological sampling team accessed the jugular vein to collect blood, obtained faecal samples from the rectum, ectoparasites and eye, nasal and oral swabs. After collection of samples, and movement of majority of the team to a safe distance, the animal was freed from the net, whilst holding the horns firmly and keeping the animal recumbent, pointing away from the net trap followed by release. On doing so, it helped to minimise injuries to animals and personnel. One animal was sampled after the other. After completion of the sampling activity, all the collected samples were labelled and stored. The period from capture to release of all the animals in the net was between 3 and 30 min depending on the group size captured. The mean time from capture and sampling to release was 17 ± 9.9. The animals were monitored for injury and mortalities for up to 8 hrs post-capture. Subsequent monitoring of the animals was done by Rangers and Maasai herdsmen residing in the area, for up to a week.

3 | RESULTS

A total of 136 Grant's and nine Thomson's gazelles were captured during the project period in three missions with an average of 18 days per mission. Evaluation of the age and sex of the animals captured by the modified net capture method shows that adult and female animals were mostly captured (Table 1). This demonstrates the behaviour of these animals in their natural habitat where adult females are in large numbers in their herds. Among the captured gazelles were three fawns which sustained hip dislocation (<2% injury rate). The fawns were inadvertently captured with the herd as separating them from adults proved problematic. These were euthanised using a recommended method described previously (Shearer & Ramirez, 2013). In numbers, the captured Grant's gazelles were 25, 44 and 67 in Loliondo, Serengeti and NCAA respectively. Of the nine-captured Thomson's gazelles, one was from Loliondo and eight from NCAA. Table 1 displays location, age category and sex of Grant's and Thomson's gazelles captured and sampled for PPR study. Table 2 displays results of comparison of the new modified technique and other adopted techniques in wildlife capture, the comparison includes four items.

TABLE 2 Method of capture and cost in finance, labour, restraint period and injury risk

S/N	Method	Cost	Person days	Rate of injury	Restraint period
1.	Net bomas	40,448	600	2%	5 min
2.	Modified Net method	20,224	300	2%	5 min
3.	Darting	54,852	287	10%	15 min

4 | DISCUSSION

Netting has become a preferred method of live capture for biologists and wildlife managers around the world when working with small to medium ungulates and some other species, due to its relatively low cost and the ability to capture larger groups of animals (Kock et al., 2012; Laubscher et al., 2015). The modified boma netting technique used in this study was an improvement on the previous operation that used a boma and drop nets when capturing a smaller number of gazelles in Ngorongoro district in the years 2014 and 2015.

There are limited reports evaluating the effectiveness of different capture methods on gazelles. Comparing with the previous methods used to capture the gazelles in these localities; the new capture method has shown improvements on aspects of efficiency, cost and safety. In the study area, each location had a calculated sample size, time allocated to accomplish capture as well as a budget which was estimated based on previous experience with different methods. With a modified netting technique, the number of animals required per location was captured in a shorter time and using a much lower budget with minimum injuries and without mortalities associated with the capture; compared with a study by Kock et al. (1987). Unlike modified netting technique where injuries were recorded only to the fawns, previous methods (the net gun, drop net, drive net and chemical immobilisation) recorded injuries, capture myopathy mortalities and accidental mortalities (Kock et al., 1987). With this technique, managers can work safely and efficiently but it is always appropriate to have veterinary staff on hand to deal with occasional injuries and to ensure the physical welfare of the animals as insisted in different studies that fear, pain, suffering and distress should be kept to a minimum (Fowler, 2003).

The modified netting technique was found to be an excellent capture technique in the rangelands of Serengeti National Park, Ngorongoro Conservation Authority and Loliondo Game Controlled areas. The main benefit of the new method was a reduction in the time spent in preparation of the capture system when compared with the previous attempts that adopted other techniques. The method proved to be equally effective with a single net line and without the use of internal drop nets, which is not the case with net boma technique. Using the modified technique the team was also able to capture Thomson's gazelles, a species that proved to be very difficult to capture in previous attempts in 2014–2015 with the use of conventional netting techniques. The Thomson's gazelles are relatively more difficult to net because of their ability to change direction of run rapidly and efficiently, avoiding capture even when running at full flight.

Ordinary netting techniques have been associated with several drawbacks. Some authors have reported limitation in use of the techniques in certain environmental conditions; and that their safety and effectiveness is not guaranteed (Sahu et al., 2017; Denicola et al., 2000; Webb et al., 1996). Authors in South Africa have associated the techniques with mortalities of the captured animals (Laubscher et al., 2015). Comparatively, the modified technique is considered to be as safe and effective in wildlife capture as the net-gun technique reported by Webb et al. (1996). However, in low-income countries, the net gun is considered costly and needs other sophisticated expertise and equipment which are not always readily available.

Comparatively, the developed technique is much more effective in terms of time and financial resources than chemical immobilisation methods which have an added disadvantage of using highly restricted and expensive drugs, and require specialist operators who are qualified veterinarians. The current cost of immobilisation chemicals for the reported number of sampled Grant's gazelles was estimated at 20 million Tanzanian shillings ($10,000) which includes cost for 10 bottles of etorphine at a concentration of 9.8 mg/ml (5 ml vials), miscellaneous sedatives, tranquilisers and antidotes; compared to 1.1 million Tanzanian shillings ($ 500) used to purchase and manufacture the netting frame used to capture gazelles by netting. For immobilisation, the budget could have been slightly low, only if almost every dart used reached the target (>98% success rate). However, darting small ruminant species is a practical challenge in cases where the flight distance is high. With gazelle species, it is not common that the darting success rate would be above an average of 30% in these conditions. In addition, the time required for fieldwork would be long, with probably a maximum of four animals per day adding further to staff costs (37 staff days immobilising versus 25 staff days netting). In addition, it is usually only feasible to dart one animal from a herd at one time and each herd is widely dispersed in this ecosystem, adding to the extra cost of vehicle fuel. Setting the net boma to capture the animals took about 2 hrs and on average seven Grant's gazelles were captured per site. The estimated cost per Grant's gazelle capture is about Tanzanian shilling 217,000 ($103) using four people for capture and sampling. Additionally, chasing of animals for immobilisation leads to extended flight during which they can either be injured or killed by inappropriate dart location on the body or suffer capture myopathy from excessive physical stress and heat. Although chasing is a component of netting too, it tends to be for a much shorter period, not physiologically very different from being chased by a predator, which normally happens almost daily with these species.

The modified net capture technique was found to be reliable for Grant's gazelle, and proved practical for Thomson's gazelles of all ages in a variety of locations in Tanzania where vehicle access was feasible. This technique proved to be safe for the operators and for the captured adult and sub-adult gazelles. The injuries observed in fawns underscore the need of avoiding inadvertently accessing fawns during wildlife capture. Although this method proved to be effective in the current environment, it may need modification in other habitats and with other species.

5 | CONCLUSIONS

Adult and sub-adult Thomson's and Grant's gazelles of both sexes can be successfully captured using the modified netting system in a variety of accessible locations in Tanzania. The system operates at a relatively lower cost and works safely when compared to chemical capture methods.

ACKNOWLEDGEMENTS

The authors would like to thank the following institutions for permission and allowing their staff to participate in one way or another during accomplishment of this task: Tanzania National Parks Authority, Ngorongoro Conservation Area Authority, Tanzania Wildlife authority, Tanzania Wildlife Research Institute, Directorate of Veterinary services and the Royal Veterinary College.

CONFLICT OF INTEREST

The authors would like to declare that they have no conflict of interest in the study.

ETHICAL APPROVAL

Note the ethics approval number: URN 2017 1741–3 –Project Title: Pathway to peste des petits ruminants virus elimination – methods for complex ecosystems Global Challenges Research Fund BB/P023002/1 Leader Royal Veterinary College University of London UK.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

ORCID

Daniel Mdetele https://orcid.org/0000-0002-6498-1835
Gerald Misinzo https://orcid.org/0000-0003-1827-6403
Erick Komba https://orcid.org/0000-0003-3242-2219
Richard Kock https://orcid.org/0000-0002-3756-8391

REFERENCES

Denicola, A. J., Vercauteren, K. C., Curtis, P. D., & Hygnstrom, S. E. (2000). *Managing white-tailed deer in suburban environments: a technical guide* (pp. 1–56). https://www.mendeley.com/catalogue/4fb07a45-c074-3451-93e9-643ec7fa2489/?utm_source=desktop&utm_medium=1.19.4&utm_campaign=open_catalog&userDocumentId=%7B3e6143cd-fd8a-495e-940c-fa6d12a5b888%7D

Farst, D. D., & Fowler, M. (2010). Restraint and handling of wild and domestic animals. *The Journal of Zoo Animal Medicine*, *10*(2), 76. https://doi.org/10.2307/20094423

Ferreira, B. P. L. (2016). *A short review of the chemical immobilization principles in some common African wildlife species*. Retrieved from https://www.repository.utl.pt/handle/10400.5/12356

Fowler, M. (2003). *Restraint and handling of wild and domestic animals* (3rd edn). Library of Congress Cataloguing (third Edit). Blackwell.

Gehr, T. (2010). Using drop nets to capture free ranging wildlife. *Newsletter*, *5*(3), 86002. https://www.wmenews.com/newsletters/wme_V5I3_En_09.pd

Hofmeyr, J. M., Luchtenstein, H. G., & Mostert, P. K. N. (1976). Capture, handling and transport of springbok and the application of haloperidol as a long-acting neuroleptic. *Madoqua*, *10*(2), 123–130.

Kock, D., Franti, E., Jessup, D. A., Weaver, R. A., & Clark, K. (1987). Capture bighorn chemical methods sheep: In five and the of free-ranging an evaluation of drop-net, immobilization. *Journal of Wildlife Diseases*, *23*(4), 634–640.

Kock, M., Meltzer, D., & Burrougts, R. (2012). *Chemical and physical restraint of wild animals: A training and field manual for African species*, 2nd ed. Greyton: IWCS. https://g.co/kgs/n3H81B

Laubscher, L. L., Pitts, N. E., Raath, J. P., & Hoffman, L. C. (2015). Non-chemical techniques used for the capture and relocation of wildlife in South Africa. *African Journal of Wildlife Research*, *45*(3), 275–286. https://doi.org/10.3957/056.045.0275

Lekolool, I. (2012). Mega-translocations : The Kenya wildlife service at its best. *The George Wright Forum*, *29*(1), 93–99. http://www.georgewright.org/291lekolool.pdf

Locke, S. L., Hess, M. F., Mosley, B. G., Cook, M. W., Hernandez, S., Parker, I. D., Harveson, L. A., Lopez, R. R., & Silvy, N. J. (2004). Ungulate Research and Management Portable drive-net for capturing urban white-tailed deer. *Wildlife Society Bulletin*, *32*(4), 1093–1098.

Mahapatra, M., Sayalel, K., Muniraju, M., Eblate, E., Fyumagwa, R., Shilinde, L., Mdaki, M., Keyyu, J., Parida, S., & Kock, R. (2015). Spillover of peste des petits ruminants virus from domestic to wild ruminants in the Serengeti ecosystem, Tanzania. *Emerging Infectious Diseases*, *21*(12), 2230–2234. https://doi.org/10.3201/eid2112.150223

Mmmalogists, A. society of. (1998). Guidelines for the capture, handling, and care of mammals as approved by the American society of mammalogists. *Journal of Mammalogy*, *79*(4), 1416–1431. https://doi.org/10.2307/1383033

Parida, S. (2017). Improvement understanding of the epidemiology of peste des petits ruminants. *(IUEPPR) Report*, *49*, 1–37. https://www.researchgate.net/publication/344610131_IUEPPR_final_report

Sahu, A. R., Wani, S. A., Saminathan, M., Rajak, K. K., Sahoo, A. P., Pandey, A., Saxena, S., Kanchan, S., Tiwari, A. K., Mishra, B., Muthuchelvan, D., Singh, R. P., Singh, Y., Baig, M., Mishra, B. P., Singh, R. K., & Gandham, R. K. (2017). Genome sequencing of an Indian peste des petits ruminants virus isolate, Izatnagar/94, and its implications for virus diversity, divergence and phylogeography. *Archives of Virology*, *162*(6), 1677–1693. https://doi.org/10.1007/s00705-017-3288-2

Shearer, J. K., & Ramirez, A. (2013). *Procedures for Humane euthanasia - Humane Euthanasia of Sick, Injured and/or Debilitated Livestock*. Retrieved from https://vetmed.iastate.edu/sites/default/files/vdpam/Extension/Dairy/Programs/HumaneEuthanasia/DownloadFiles/EuthanasiaBrochure20130128.pdf

Webb, S. L., Lewis, J. S., Hewitt, D. G., Hellickson, M. W., & Bryant, F. C. (1996). Assessing the helicopter and net gun as a capture technique for white-tailed deer. *Journal of Wildlife Management*, *72*(1), 310–315. https://doi.org/10.2193/2007-101

How to cite this article: Mdetele D, Misinzo G, Komba E, et al. Modified netting technique for capturing gazelles in Serengeti, Ngorongoro and Loliondo, Tanzania. *Afr J Ecol*. 2020;00:1–7. https://doi.org/10.1111/aje.12813

Manuscript V

A Comparative study of Peste des petitis Ruminants Virus Sero-prevalence among Wildlife from different habitats of the Serengeti Ecosystem, Tanzania

[1]Mdetele, D.[*], [3]Mdaki, M., [1]Makange M.R, [3]Elbret, E., [3]Shemanche, J., [4]Kabululu, M., [1]Misinzo, G., [3]Keyyu, J., [1]Komba, E., [2]Jones, B. and Kock, R.

[1]SACIDS Foundation for One Health, Sokoine University of Agriculture, P.O. Box 3297, Chuo Kikuu, Morogoro.

[2]Royal Veterinary College, Hawkshead Lane, Hatfield, Herts AL9 7TA, UK

[3]Tanzania Wildlife Research Institute, P.O Box 661, Arusha, Tanzania.

[4]Tanzania Livestock Research Institute (TALIRI) - Uyole, P.O. Box 6191, Mbeya, Tanzania

Email: daniel.mdetele@sacids.org

In preparation

A Comparative study of Peste des petitis Ruminants Virus Sero-prevalence among Wildlife from different habitats of the Serengeti Ecosystem, Tanzania

[1]Mdetele, D.[*], [3]Mdaki, M., [1]Makange M.R, [3]Elbret, E., [3]Shemanche, J., [4]Kabululu, M., [1]Misinzo, G., [3]Keyyu, J., [1]Komba, E., [2]Jones, B. and Kock, R.

[1]SACIDS Foundation for One Health, Sokoine University of Agriculture, P.O. Box 3297, Chuo Kikuu, Morogoro.

[2]Royal Veterinary College, Hawkshead Lane, Hatfield, Herts AL9 7TA, UK

[3]Tanzania Wildlife Research Institute, P.O Box 661, Arusha, Tanzania.

[4]Tanzania Livestock Research Institute (TALIRI) - Uyole, P.O. Box 6191, Mbeya, Tanzania

Email: daniel.mdetele@sacids.org

Abstract

Peste des petits ruminants (PPR), is a severe transboundary viral animal disease of domestic and wild small ruminants, causing mortalities that threaten food security and conservation in Africa and Asia. Outbreaks of the disease have been confirmed in wild species in Middle East and Asia but not in Africa. The objective of this study was to determine the spill over of PPR virus, found and confirmed in livestock living in closer proximity to wildlife from different habitats of Serengeti ecosystem in Tanzania. A cross-sectional survey was conducted to determine the seroprevalence of PPR in wildlife species. Four wildlife species buffalos (*Syncerus caffer*), impalas (*Aepyceros melampus*), Grant's gazelles (*Nanger granti*) and Thomson's gazelle (*Eudorcas thomsonii*), of different ages and sex were sampled from different zones (habitats). Wildlife sampling was done in the following zones; Serengeti National Park (SNP), Ngorongoro Conservation Area Authority (NCAA) and Loliondo Game Controlled Area (LGCA). Sample sizes were calculated based on wildlife population according to Tanzania Wildlife Research Institute (TAWIRI) aerial census. Capture of gazelles was by netting while buffalos and impala were captured by chemical immobilization. Blood samples were collected for PPRV antibody detection using competitive Enzyme-linked immunosorbent assay (c-ELISA) technique. A total of 270 wildlife were captured, 26 (9.6%) from LGCA, 75 (27.8%) from NCAA and 169 (62.6%) from SNP, out of which two (7.7%), seven (9.3%) and 30 (17.8%) were seropositive, respectively. Results for one (3.8%), six (8%) and 42 (24.8%) animals from LGCA, NCAA and SNP, respectively, were doubtful. There were no statistically significant differences in seropositivity between zones, species, age categories and sex. PPR virus sero-convesion in wildlife species indicate that wildlife have been exposed to the PPRV. Having no statistical significant different in seroprevalence among wildlife species from different habitats indicated the magnitude of virus circulation among wildlife from different habitat is the same.

Key words: PPR, Sero-prevalence, Wildlife, co-existence and Serengeti ecosystem

Introduction

Peste des petits ruminant (PPR) is a highly transmissible viral disease mostly affecting sheep and goats in Africa, Asia and Middle East. As well, it has been affecting camels and wildlife causing high morbidity and mortalities in naïve population, resulting into loss of meat and milk to livestock keepers. This results into threatening food security and livelihoods of communities depending on small ruminants (Kihu and Gitao *et al.,* 2015). Moreover, in wildlife, it threatens conservation as outbreaks have been confirmed in wild species in the middle East and Asia though not yet confirmed in Africa including Tanzania (Kock *et al.,* 2015; Pruvot *et al.,* 2020; Shatar *et al.,* 2017). In Tanzania, PPR antibodies have been detected in wildlife which is associated with PPR spill over from the infected livestock (Mahapatra *et al*., 2015). The role of wildlife in the spread and maintenance of PPR is not clearly known at the moment. An outbreak of PPR has been reported in livestock that share grazing, watering and salt licking areas with wildlife. Likewise, PPR outbreaks have been reported and confirmed in free ranging wildlife elsewhere, where wildlife were sharing grazing with livestock which had experienced PPR outbreaks, as well as in the confined wildlife in zoos (Pruvot *et al.,* 2020). In Tanzania, purposive sampling was conducted in 2014-15 in wildlife co-existing with livestock in the Serengeti ecosystem, where PPR antibodies were detected in buffalos (*Syncerus-caffer*), wildebeests (*Connochaetes taurinus*), Grant's gazelles (*Nanger-granti),* topis (*Damaliscus lunatus jimela*), hartebeests (*Alcelaphus buselaphus*) and impalas (*Aepyceros melampus*) (Mahapatra *et al.,* 2015). PPR outbreaks were confirmed in sheep and goats in adjacent areas of Ngorongoro district during the same period. PPR virus (PPRV) is endemic in Tanzania and most of Africa, Asia and threatens Europe (Parida *et al.,* 2016). It has

become a constraint to small ruminant production, trade, welfare and threatens conservation of endangered wildlife species co-existing with livestock (Kock *et al.*, 2015; Munir, 2014a, 2014b; Travis *et al.*, 2011). The objective of this study was to investigate the exposure of wild animals to PPRV and establish the role of wildlife in maintenance and spread of PPR in the complex ecosystems. The generated information is necessary for developing measures which limit further spread and maintenance of PPRV in Tanzania.

Methodology

Study area

The study was carried out in Serengeti ecosystem located in northern Tanzania, with an area spanning approximately 30 000 km^2 including Serengeti National Park (SNP), Ngorongoro Conservation Area Authority (NCAA) and Loliondo Game Controlled Area (LGCA) (Figure 1). SNP is an area designated only for wildlife, while in NCAA wildlife and livestock co-exist but other human activities are prohibited. In LGCA wildlife and livestock co-exist and other human activities are allowed. SNP covers 14 750 km^2 of grassland plains, savannah, riverine forest and woodlands. It is found in northwest Tanzania, bordered to the north by the Maasai Mara National Reserve of Kenya, NCAA to the southeast, Maswa Game Reserve to the southwest, Ikorongo and Grumeti Game Reserves to the west and to the northeast and east is LGCA.

Ngorongoro Conservation Area Authority is a protected area and a World Heritage, where on the East it borders with Karatu district, on the west borders with SNP, on the South borders with Meatu district and on the Northern side borders with LGCA. The area is named after Ngorongoro Crater, a large volcanic caldera within the area. The conservation area is administered by the NCCA.

Loliondo Game Controlled Area which covers an estimated area of 4000 km^2 is a unique area providing mixed activities for the community. The main land use in LGCA is livestock keeping comprising of pastoralists in about 90% of the area, wildlife conservation, small scale agriculture and tourism - hunting of wild animals. SNP, NCAA and LGCA together form the Serengeti ecosystem.

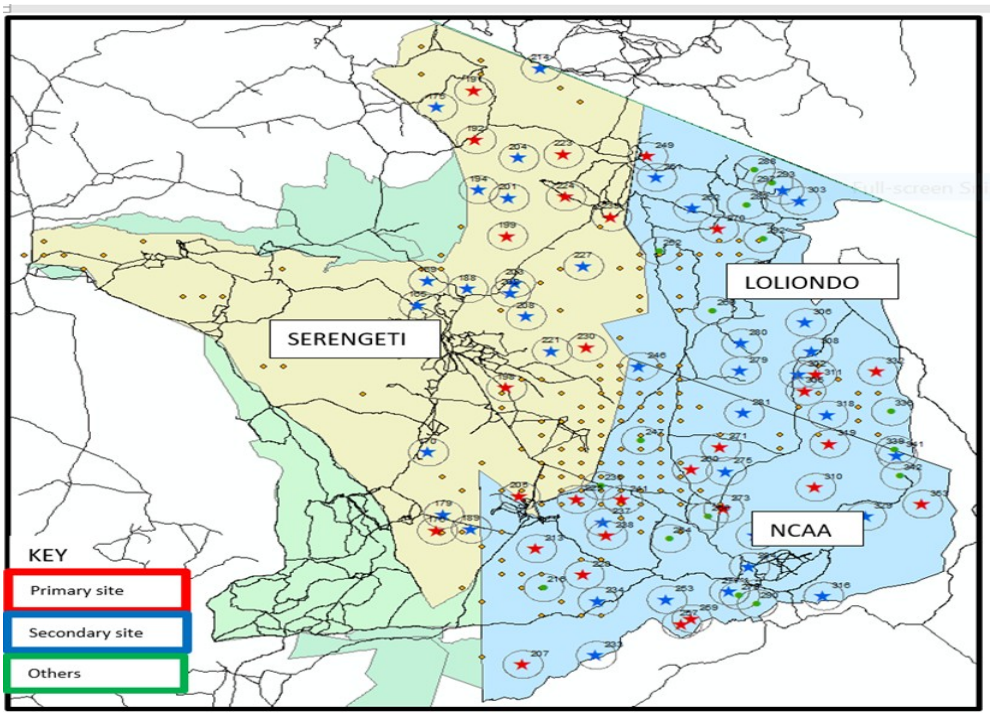


Figure 1: Map of Serengeti ecosystem showing Serengeti National Park (SNP), Ngorongoro Conservation Area Authority (NCAA) and Loliondo Game Controlled Area (LGCA). Showing; primary, secondary and other sampling points

Sampling sites and procedure

Sample size was calculated based on wildlife population, information obtained from TAWIRI aerial census of 2009 and 2010. PROMESA software [http://www.promesa.co.nz/ProMESA.htm] was used to estimate sample size per location. Each sampling point was assigned with the geo-referenced point, where animals that were found within a radius of 5 km from the center point of selected geo-reference were captured using chemical

immobilization or modified netting technique. From each sampling point, around five animals were sampled. Sampling was done in four rounds, First round during 23rd Aug – 5th September 2018 and Second round during 17th – 24th October 2018, third round during 5th - 25th March 2019 and fourth round during 15th - 24th August 2019. Immobilization of buffalo's adopted the use 9 - 12 mg etorphine in combination with100 mg xylazine or 50 - 80 mg Azaperone.

Immobilization was reversed by 18 mg diprenorphine. The immobilization of Impala was achieved by a combination of 3 mg etorphine, 3 mg meditomidine and 15 mg azaperone; and reversal was attained by 8 mg naltrexone and 1mg yohimbine. Modified netting technique was used for Grant's and Thomson's gazelles. Initial sampling started on primary sampling points (red points on Figure 1) which were featured by good terrain. These were followed by secondary points (blue points) and finally other sampling points were used (green points). The exclusion criteria for sampling points were bad terrain and limited number of animals.

Sample collection

Blood was collected from the jugular vein in three 5 mL plain vacutainers and one 5 mL vacutainer with EDTA. The blood in plain vacutainer was left to settle over night before extracting the serum in 2.5 mL cryovials in the next day. Extracted serum samples were stored frozen (-20^{o}C) temporarily at SNP laboratory before they were transported to TAWIRI head office laboratory in Arusha where they were stored at -20^{o}C, From Arusha the samples were transported to Sokoine University of Agriculture (SUA) and were stored at -80^{o}C.

Laboratory testing

The laboratory analysis of collected samples employed the c-ELISA technique using the ID screen®, a competitive ELISA kit (IDvet, Grabels, France) for the detection of anti-PPRV nucleoprotein antibodies in serum or plasma. The kit designed for detection of antibodies directed against the nucleoprotein of the PPRV. The test uses a plate coated with purified recombinant PPR nucleoprotein (NP). The working principle of the kit is that, anti-NP antibodies if present form an antibody-antigen complex which masks in the NP epitopes. Anti-NP-peroxidase conjugate fixes to the remaining free NP epitopes, forming an antigen-conjugate-HRP complex. On washing the plate, the excess conjugate is eliminated. The substrate solution results into coloration depending on the quantity of specific antibodies present in the sample to be tested. In the absence of antibodies, blue solution appears which becomes yellow in addition of stop solution. In presence of antibodies, no coloration appears.

The following procedure was followed; all reagents were allowed to come to room temperature and were homogenised by inversion. Micropipette calibrated at 25 µL was used to add dilution buffer 13 to wells. Then 25 µL of the positive controls were added to wells A1 and B1 which was followed by adding 25 µL of Negative controls to wells C1 and D1. Finally 25 µL of each sample was added to the remaining wells. The plate was incubated for 45min ± 4min at 37ºC, which was followed by washing of each well three times using 300 µL of washing solution. Then conjugate 1X prepared by diluting conjugate 10x to 1/10 in dilution buffer 4 was added to each well using micropipette calibrated at 100 µL and incubated for 30min at 21ºC (±5ºC). Again each well was washed three times using 300 µL of the wash solution. This was followed by addition of 100 µL of the substrate solution to each well and was incubated for 15min ±2min at 21ºC (±5ºC) in the dark. Finally 100 µL of the stop solution added to each well in order to stop the

reaction. Later microplate leader was used to read, at the Optical Densities (OD) of 450 nm.

The results for each sample were calculated based on the competition percentage (S/N %), Where S/N% was calculated by taking sample OD value and divide it by OD value of negative control times one hundred. Where S/N% was ≤ 50% the sample was considered as positive, 50% < S/N% ≤ 60% was considered doubtful and S/N %>60% considered as negative (Libeau *et al.*, 1995).

Data analysis

The data were analysed using STATA© Version 12 computer software. Descriptive statistics were computed for determining frequencies and proportions. Multivariate logistic regression analysis was performed to assess association of zones (habitats), species and age category variables with serological status. Since the cut off points for positive and negative serological results were <50 and >60 respectively based on test kit manufacturer's guidance, all the doubtful <55 were considered positive and those >55 were considered negative during analytic analysis.

Results

Table 1: Sero-prevalence of PPR among different wildlife species in the Serengeti ecosystem, Tanzania

Results	Grant's gazelle	Thompson's gazelle	Buffalo	Impala	Total
Doubtful	14 (10.3%)	0	32 (28.8%)	3 (21.4%)	49 (18.2%)
Negative	110 (80.9%)	6 (66.7%)	55 (49.6%)	11 (78.6%)	182 (67.4%)
Positive	12 (8.8%)	3 (33.3%)	24 (21.6%)	0 (0%)	39 (14,4%)
Total	136	9	111 (41.1%)	14 (5.2%)	270

Table 2: Sero-prevalence of PPR among different zones (habitats) in the Serengeti ecosystem, Tanzania

Results	LGCA	NCAA	SNP	Total
Doubtful	1 (3.8%)	6 (8%)	42 (24.8%)	49 (18.2%)
Negative	23 (88.5%)	62 (82.7%)	97 (57.4%)	182 (67.4%)
Positive	2 (7.7%)	7 (9.3%)	30 (17.8%)	39 (14.4%)
Total	26	75	169	270

Table 3: Multivariate logistic regression analysis to determine association of zones, species, age categories and sex with PPR serological status

Variables		Odds Ratio	Std. Error	Z	P>/Z/	95% conf. interval	
Zone	SNP	0.6309	0.4397	0.66	0.509	0.16096	2.47265
	LGCA	1.3762	0.6859	0.64	0.522	0.51818	3.65512

	NCAA	1			-		
Species	Buffaloes	0.679815	0.311165	0.84	0.399	0.27719	1.66726
	Impala	0.560481	0.477942	0.68	0.497	0.105369	2.98133
	Thomson’s gazelles	0.680222	0.759151	0.35	0.730	0.076328	6.06207
	Grant’s gazelles	1			-		
Age	Young	1.292667	1.110021	0.30	0.765	0.2401899	6.95695
Category							
	Sub-adult	1.099303	0.4545546	0.23	0.819	0.4888223	2.47220
	aged	1.842011	0.9864555	1.14	0.254	0.6448156	5.26198
	Adult	1			-		
Sex	Male	1.246387	0.4089248	0.67	0.502	0.6552145	2.37095
	Female	1				-	

A total of 270 wildlife were captured, 26 (9.6%) from LGCA, 75 (27.8%) from NCAA and 169 (62.6%) from SNP (Table 2). The c-ELISA test showed that 39 (14.4%), 49 (18.2%) and 182 (67.4%) of the sampled wildlife had positive, doubtful and negative results, respectively (Table 2). Two (7.7%), seven (9.3%) and 30 (17.8%) animals were seropositive from LGCA, NCAA and SNP respectively. Doubtful results were recorded for one (3.8%), six (8%) and 42 (24.8%) animals from LGCA, NCAA and SNP respectively (Table 2). Compering PPR seroprevalence among different species of wild animals captured, sampled and analysed: Grants Gazelles, Thomson gazelles, Buffaloes and Impala the seropositivity were 12 (8.8%), 3 (33.3%), 24 (21.6%) and 0 (0%) respectively (Table 1). Statistically there were no significant difference in seropositivity from animals coming from different zones/habitat meaning that no difference between animals sampled from areas where there was co-existence of wildlife and livestock with no other human activities, wildlife only area and area with wildlife, livestock plus other human activities.

Discussion

Although PPR is known as a sheep and goat disease, clinical cases in domestic animals other than sheep and goats, wildlife species have been reported and confirmed (Khalafalla *et al.,* 2010; Kock *et al.,* 2015; Shatar *et al.,* 2017). PPR outbreaks in species other than sheep and goats have been reported in areas where affected livestock share grazing and watering areas with clinically infected sheep and goats (Intisar *et al.,* 2017; Pruvot *et al.,* 2020). During this study, there was no clinical case of PPR that was noted among wildlife found in the study area. However, collected sera samples from some wildlife species were found to be PPR seropositive. These sero-converted animals confirm that, they had been in contact with the PPRV. Compared with livestock from the same area, the seroconversion rate in wildlife is slightly lower (Jones *et al.,* 2020).

It has been hypothesized that, there is spill over of the virus from infected domestic small ruminants to wildlife (Mahapatra *et al.,* 2015). The sero surveys undertaken in wild animals prior to the introduction of PPR in livestock were found to be seronegative confirming that wild animals has been into contact with the virus that resulted into diseases outbreak in livestock (Taylor *et al.,* 2002; Lembo *et al.,* 2013). The seroconversion reported in the current study among the captured and sampled wildlife species from three different zones (habitats) and all species confirm the spill over. These seroconverted animals from different species must have come into contact with PPRV at one point in time. The sero surveys conducted in wildlife earlier before first confirmation of PPR in livestock in Tanzania found that the wildlife were seronegative (Kock *et al.,* 2006) *(*Lembo *et al.,* 2013). Since the first clinical cases of PPR were found and confirmed in Tanzania, subsequent cases were found in livestock which are found in close proximity to wildlife, especially in LGCA and NCAA in Ngorongoro district (Kivaria *et al.,* 2013). Therefore, this study proves the fact that sero-conversions observed in wildlife have their origin from infected livestock in the area. Pest des petits ruminants has been confirmed in sheep and goats found in NCAA at different periods (Jones *et al.,* 2020; Kgotlele *et al.,* 2014b; Mahapatra *et al.,* 2015). Similarly, during this study PPR outbreaks were confirmed in livestock found in LGCA, NCAA and Karatu districts. The livestock from these areas are co-existing with wildlife; and wildlife in these areas share grazing, watering and salt leaking areas with the livestock confirmed to have PPR. Therefore, it is evident that the sero-conversion observed in wildlife is due to virus spill over from livestock to the wild animals. Though there was no observed PPR clinical case in the wildlife during the study period, chances are that, such clinically sick wild animals get predated on by carnivorous as they get weak and easily spotted. A sick animal becomes weak and an easy target for carnivores in these kind of ecosystems where pray and predators co-exist. In that way it is difficult to observe such animals in one or two

visits. Likely, if clinically sick animals consumed by carnivores at an early stage of disease, such a situation tends to limit further disease transmission either among wild animals or back to livestock that share grazing resources. In that way, less wild animals were found to have PPR antibodies and no PPR clinical cases were observed among wildlife in the Serengeti ecosystem. Hence, PPR becomes a self-limiting disease in a complete ecosystem like Serengeti where prey and predators are found in an ecologically balanced ratio.

There was no a statistically significant difference when a comparison was made for the magnitude of seroconversion between wildlife found in closer proximity to livestock (NCAA and LGCA) and those found far away or not in contact at all with livestock (SNP). Normally, it was expected that wildlife coexisting with livestock could have a higher level of sero-conversion in case the transmission is limited only from livestock to wildlife as it has been explained in other studies (Mahapatra *et al.,* 2015). Nevertheless, wildlife species from areas where there is no interaction with livestock were found to have equally seroconverted. The findings suggest that there is possibility of PPR being cross transmitted from livestock to wildlife and equally the infected wildlife transmit to other wildlife that are not coming into contact with livestock, though this cannot rule out the fact that various studies have shown that livestock have been entering illegally in the protected areas especially during drought period searching for water and grazing pasture (Mdetele *et al.*, 2015), in that way transmitting PPR to wildlife in areas where livestock are not found legally. As well during the interview with the residents, they mentioned that the practise of taking animals to the protected areas is common among livestock keepers surrounding those areas.

The low PPR seroprevalence in the NCAA, together with co-existence of wildlife and livestock, could have been influenced by the conservation authority investing on PPR routine procurement and free vaccinations to sheep and goat herds found within NCAA area. This practise limits PPR virus spread among livestock and spilling over to the wildlife, unlike in the LGCA where there is no properly organised vaccination of livestock to control PPR. As well unvaccinated animals grazing around the SNP and animals entering illegally in the SNP could have resulted into relatively higher PPR seroprevalence in the SNP and LGCA, resulting into the two areas having a relatively higher seroprevalence compared to the NCAA.

Limitation of the study

The c-ELISA test kits used in this study for analysis of samples have been validated for goat and sheep samples, and not other species. This could have resulted into high variations between the positive, negative and doubtful results. Therefore, a need for validation of the test kits for wildlife and other species is needed.

Conclusion and Recommendations

Findings of this study suggest that there is spill over of PPR virus from livestock to wildlife, affected livestock spill the virus to the wild animals. The control of PPR in livestock limits spread of the disease to wildlife coexisting with livestock. Therefore, need further study to establish possibility of virus being maintained and spread back to the livestock. Furthermore, there is a need for the validation of the c-ELISA test kit for wildlife as the percentages of doubtful results were higher.

Acknowledgements

The authors would like to acknowledge the Royal Veterinary College - London, Sokoine University of Agriculture, local government authorities Serengeti and Ngorongoro districts, Directorate of Veterinary services - Tanzania, Tanzania Wildlife Research Institute, Pirbright Institute and SACIDS Foundation for One Health for making this work possible. The Global Challenges Research Fund of the UK government is acknowledgment for providing support for the research titled, Pathway to Peste des Petits Ruminants Virus Elimination - methods for complex ecosystems PPR, in a mixed wildlife livestock ecosystem in East Africa BB/P023002/1. The support was extended through the Biotechnology and Biological Sciences Research Council.

References

Intisar, K. S., Ali, Y. H., Haj, M. A., Sahar, M. A. T., Shaza, M. M., Baraa, A. M. and Diallo, A. (2017). Peste des petits ruminants infection in domestic ruminants in Sudan. *Tropical Animal Health and Production* 49(4): 747–754.

Jones, B. A., Mahapatra, M., Chubwa, C., Clarke, B., Batten, C., Hicks, H. and Parida, S. (2020). Characterisation of peste des petits ruminants disease in pastoralist flocks in Ngorongoro district of northern Tanzania and bluetongue virus co-infection. *Viruses* 12(4): 1 - 14.

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

Khalafalla, A. I., Saeed, I. K., Ali, Y. H., Abdurrahman, M. B., Kwiatek, O., Libeau, G. and Abbas, Z. (2010). An outbreak of peste des petits ruminants (PPR) in camels in the Sudan. *Acta Tropica* 116(2): 161–165.

Kihu, S. M., Gitao, G. C., Bebora, L. C., Njenga, J. M., Wairire, G. G., Maingi, N. and Wahome, R. G. (2015). "Economic losses associated with Peste des petits ruminants in Turkana County Kenya," *Pastoralism* 5(1): 96 - 99.

Kivaria, F. M., Kwiatek, O., Kapaga, A. M., Swai, E. S., Libeau, G., Moshy, W. and Gladson, J. (2014). The incursion, persistence and spread of peste des petits ruminants in Tanzania: Epidemiological patterns and predictions. *Onderstepoort. Journal of Veterinary Research* 81(1): 1 - 10.

Kock, R. A., Orynbayev, M. B., Sultankulova, K. T., Strochkov, V. M., Omarova, Z. D., Shalgynbayev, E. K. and Parida, S. (2015). Detection and Genetic Characterization of Lineage iv Peste Des Petits Ruminant Virus in Kazakhstan. *Transboundary and Emerging Diseases* 62(5): 470 - 479.

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

Mahapatra, M., Sayalel, K., Muniraju, M., Eblate, E., Fyumagwa, R., Shilinde, L. and Kock, R. (2015). Spillover of Peste des Petits Ruminants Virus from Domestic to Wild Ruminants in the Serengeti Ecosystem, Tanzania. *Emerging Infectious Diseases* 21(12): 2230 – 2234.

Mdetele, D. C., Kasanga, C., Seth, M. and Kayunze, V. (2015). “Socio-economic Impact of Foot and Mouth Disease in Wildlife- Livestock Interface and Non-Interface of Tanzania,” *World s Veterinary Journal* 6(1): 31 - 35.

Munir, M. (2014). Role of wild small ruminants in the epidemiology of peste des petits ruminants. *Transboundary and Emerging Diseases* 61: 411- 424.

Parida, S., Muniraju, M., Altan, E., Baazizi, R., Raj, G. D. and Mahapatra, M. (2016) 'Emergence of PPR and its threat to Europe', *Small Ruminant Research* 142: 16–21.

Pruvot, M., Fine, A. E., Hollinger, C., Strindberg, S., Damdinjav, B., Buuveibaatar, B., and Shiilegdamba, E. (2020). Outbreak of peste des petits ruminants virus among critically endangered mongolian saiga and other wild ungulates, Mongolia, 2016-2017. *Emerging Infectious Diseases* 4(26): 1 - 9.

Shatar, M., Khanui, B., Purevtserem, D., Khaishgee, B., Loitssh, A., Unger, H., Settpalli, T. D., Cattoli, G., Damdinjav, B. and Dungon, W.G. (2017). “First genetic characterization of peste des petits ruminants virus from Mongolia,” *Archieves of Virology* 162(10): 3157 - 3160.

Travis, D. A., Watson, R. P. and Tauer, A. (2011). The spread of pathogens through trade in wildlife. *OIE Revue Scientifique et Technique* 30(1): 219–239.

CHAPTER THREE

3.0 General Discussion

PPR was confirmed in 2008 at northern Tanzania, since then it has been spreading from northern part to other part of the country. At the moment is considered to be endemic in the country. It is likely to have been introduced from Narok, Kenya into northern Tanzania through cross-border movement of small ruminants for trade or for grazing resources and then spread within Tanzania through the movement of small ruminants by pastoralists and traders during 2008 to 2010. Presence of PPR virus lineages II, III and IV indicates that there has been more than one phase of virus introduction in the country. Currently there is no evidence of PPR clinical disease in wildlife species, although serological samples from several wild ruminant species indicate sero-conversion and therefore previous exposure with PPR virus is evident. Similarly, no PPR disease has been observed in cattle and camels, but serological evidence indicates exposure to PPR virus infection among these animal species.

The epidemiological study of PPR virus in ecosystems with populations of multiple in-contact susceptible wild and domestic ruminant species suggests a zonal predisposition of PPR sero-prevalence with districts in the semi-arid and coastal zones having significantly higher values compared to those in the plateau ecological zones. Analysis of 2490 samples from sheep and goats confirmed the endemic nature and wide distribution of PPR throughout Tanzania. The findings which are in line with what has been reported in previous studies carried out in the country, where PPR sero-prevalences were found to be higher in arid and semi-arid districts of Ngorongoro, Longido, Simanjiro and Monduli (Swai *et al.*, 2009; Kivaria *et al.*, 2014), as well as coastal districts of Lindi and Mtwara

(Muse *et al.,* 2012; Kivaria *et al.,* 2014; Franklin *et al.,* 2015). Similarly, in other parts of the East African region, studies have shown PPR sero-prevalences to be higher in arid and semi-arid areas. These include studies conducted in Turkana and Karamoja in Kenya and Uganda, respectively (Ruhweza *et al.,* 2010; Gitao *et al.*, 2014; Kihu *et al.,* 2015; Dundon *et al.,* 2017; Nkamwesiga *et al.,* 2019). In Nigeria, sero-survey of PPRV in small ruminants from different ecological zones showed that the states found on savannah areas, which are ecologically similar to arid and semi-arid zones, had higher PPR sero-prevalence compared to states in the tropical rain forest and plateau (Woma *et al.,* 2016). According to Lefèvre and Diallo (1990), PPRV infections persist in regions of low relative humidity and that the PPRV survives longer in dry regions (Morandell *et al.,* 2008). The mentioned attributes are a characteristic of the zones found to have higher sero-prevalence in this study and therefore their observations are in support of the findings of the current study.

On the characterization of PPR disease in an area where livestock interact with wildlife in the Serengeti ecosystem (Karatu, Longido, Meatu, Monduli, Ngorongoro and Serengeti districts). PPR was confirmed in areas where clinical cases were reported by livestock field officers in small ruminant populations from different localities. Clinical signs observed and used for cases identification and later verified by rapid field test (LFD) and c-Elisa test in the laboratory were similar to those reported in other studies; both in the country and elsewhere (Muse *et al.,* 2012; Kgotlele *et al.,* 2014; Jones *et al.,* 2020). Moreover, qPCR was used to confirm the diagnosis. PPR cases were confirmed in all divisions of Ngorongoro district, the epicentre of greater Serengeti Ecosystem area and Karatu district in the ward bordering NCAA. Most confirmed cases were among young sheep and goats coexisting with wildlife. The lineage III was found to be prevailing lineage in the area. The disease presented itself with mild clinical signs unlike in a naïve

population where the disease is normally severe and occurs in form of outbreaks. The pastoralists were found to be aware of the disease clinical signs, even though in some cases they confused with other diseases with similar clinical manifestation. The distribution of PPR confirmed cases in the study area suggested higher chances of PPR spilling over to wildlife as several confirmed cases occurred in areas where livestock co-exist with wildlife. In addition, to that other confirmed cases were found in the country's border villages. A participatory map drawn by villagers indicated that animals from villages in Kenya and Tanzania share grazing and salt leak resources. In that case there were higher chances for the disease moving between two countries, something which can complicate the control if there is no cross border agreements on joint surveillance and control of the diseases.

On comparing PPR sero-conversion rates between wildlife co-existing with PPR infected livestock to those which are not in close contact with livestock to determine if there is spill over of PPR from infected livestock to wildlife, animals from all habitat found to have sero converted. This is the first time that ppr seropositivity is reported in wide variety of habitat. Therefore, the finding of this study suggests that there is spill over of PPRV from infected livestock to wildlife. On comparing the sero – conversion rate between habitat/zones, species and age categories it was found that there were no statistically significant difference among groups of animals from those different habitats, species and age categories. Normal it was expected that wildlife co-existing with livestock would have higher percentage of sero-conversion between habitats/zones. Relatively, animals from areas where there is no interaction found to have higher percentage on sero prevalence than areas where wildlife co-exists with livestock. The assumption is there is possibility of the PPRV being cross-transmitted from livestock to wildlife and from infected wildlife to other wildlife which has not come into contact with livestock. Although no PPR clinical

case observed among wild animals during study period, chances are such animals are being consumed by carnivores. In areas with complete ecosystem like Serengeti where number of herbivore and carnivores are checked and balanced by nature there possibilities such animals with clinical signs are easily spotted and get consumed by carnivorous, something which results into not observing sick wild animal easily. The study area is a wilderness where as soon as an animal gets sick, it is easily spotted by carnivores and considered as a weak pray, therefore it can be very difficult to find such animals in one or two visits. The finding does not suggest on either the sero-conversion was caused by interaction with infected livestock or interaction among infected wildlife and health animals. On comparing different zones/habitats there was no statistically significant difference among wildlife that coexist with livestock and areas where there is no co-existence. However, chances are livestock are source of the virus even in the non-co-existence areas due to the fact that various studies have shown that livestock have been entering illegally in the protected areas especially during drought periods searching for water and grazing pasture (Mdetele *et al.*, 2015). During interviews with the residents, they also mentioned that the practise of taking animals to the protected areas is common among livestock keepers surrounding those areas. In addition study has shown large disparity between positive, negative and doubtful results in the serological tests between species as well as between zones/habitats. Such disparity necessitate for validation of c-Elisa for wild animals and other species apart from sheep and goats.

CHAPTER FOUR

4.0 General Conclusion and Recommendations

4.1 Conclusion

Since entry of PPR in the country on northern Tanzania in 2008, the disease has been spreading and maintaining all over the country despite some efforts which have been used to control it. Pastoralist and trade stock movements have been found to be the main reason for the disease spreading and maintenance, lack of proper surveillance and control strategies by vaccination is the other reasons for the existence of the disease into different parts of the country. PPR distribution in the country shows agro-ecological zone pre-disposition, with sero-prevalences being higher in semi-arid and coastal agro ecological zones associated with pastoralist and live animals traders movements.

The disease has been confirmed in the Serengeti Ecosystem area, the confirmed cases were among young sheep and goats coexisting with wildlife, as well other confirmed cases were depicted on the cross border villages. The lineage III was found to be a prevailing lineage in the area. Currently the disease has been presenting with mild clinical signs unlike in a naïve population where the disease is normally severe and occurs in form of outbreaks. It has been found that pastoralists are aware of the disease clinical signs, even though in some cases confused with other diseases with similar clinical manifestation.

No PPR clinical case has been found in wildlife. However, all investigated species from all zones/habitats were found to have Sero-converted. Wild animal sero conversion indicate the spilling over of PPRV from infected livestock to wildlife, studies done before PPR introduction to Tanzania found no sero - conversion. Nevertheless, the number of the

sero-positive and doubtful results among species and habitat was high, a thing which need to consider validation of the c-ELISA test kit for wildlife species, other animals apart from sheep and goats.

4.2 Recommendations

i. Control or eradication strategies for PPR should consider the agro ecological zones, suggestive of the favourable conditions for survival and perpetuation of the virus among reservoir hosts and the susceptible animal populations in these areas.

ii. Pastoralists, agro pastoralists and live animals traders of live animal are among key stakeholders to be engaged both in development and implementation PPR surveillance, disease prevention, control and eradication strategies.

iii. PPR surveillance and control in conserved areas need a joint effort between the Veterinary and conservation authorities. In the cross border districts strategies need cross-border agreements and harmonization.

iv. Considering high percentage of doubtful results observed from the wild life samples subjected to c-ELISA diagnsosis as compared to livestock samples, there is a need for PPR c-ELISA test kits validation for species other than sheep and goats.

References

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

Abu Elzein, E. M. E., Hassanien, M. M., Alfaleg, A. I. A., Abd Elhadi, M. A. and Housawi, F. M. T. (1990). Isolation of PPR virus from goats in Saudi Arabia. *Veterinary Receive* 127: 309 - 315.

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

Anderson, J. and Mckay, J.A. (1994). The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implication to rinderpest control programmes. Epidemiol. *Infected* 112: 225–234.

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

Balamurugan, V., Sen, A., Venkatesan, G., Yadav, V., Bhanot, V., Bhanuprakash, V. and Singh, R.K. (2010). Application of semi-quantitative M gene-based hydrolysis probe (TaqMan) real-time RT-PCR assay for the detection of Peste des petits ruminants virus in the clinical samples for investigation into clinical prevalence of disease. *Transbound Emerg Diseases* 57: 383–395.

Balamurugan, V., Krishnamoorthy, D.S.N., Raju, K.K., Rajak. K. and Bhanuprakash, V. (2014). Prevalence of Peste-des-petits-ruminant virus antibodies in cattle, buffaloes, sheep and goats in India. *Virus Disease* 25: 85 - 90.

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

Bao, J., Li, L., Wang, Z., Barrett, T., Suo, L., Zhao, W., Liu, Y., Liu, C. and Li, J. (2008). Development of one-step real-time RT-PCR assay for detection and quantitation of peste des petits ruminants virus. *Journal of Virology Methods* 148: 232–236.

Baron, J., Bin-Tarif, A., Herbert, R., Frost, L., Taylor, G. and Baron, M. D. (2014). Early changes in cytokine expression in peste des petits ruminants disease. *Veterinary Research* 45(1): 56 – 89.

Barrett, T. (2001). Morbilliviruses: dangers old and new. In: Smith, G.L., Mc Cauley, J. W., Rowlands, D. J. (Eds.), New Challenges to Health: The Threat of Virus

Infection: Society for General Microbiology, Symposium 60. Cambridge University Press. pp. 155-178.

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

Batten, C. A., Banyard, A. C., King, D. P., Henstock, M. R., Edwards, L., Sanders, A., Buczkowski, H., Oura, C. A. L. and Barrett, T. (2011). A real-time PCR assay for the specific detection of peste des petits ruminants virus. *Journal of Virololgy Methods* 171 (2): 401– 404.

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

Choi, K.S., Nah, J.J., Ko, Y.J., Kang, S.Y. and Jo, N.I. (2005). Rapid competitive enzyme-linked mmunosorbent assay for detection of antibodies to peste des petits ruminants virus. *Clinical Diagnosis Laboratory Immunology* 12: 542–547.

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

Diallo, A. (1988). Peste bovine et peste des petits ruminants: des menaces constantes contre l'élevage dans beacoup de pays en development. Impact: *Science et Société* 150: 191- 204.

Diallo, A. (1990). Morbillivirus group: genome organization and proteins. *Veterinary Microbiology* 23: 155-163.

Diallo, A., Minet, C. and Goff, C. (2007). The threat of peste des petits ruminants: progress in vaccine development for disease control. In Vaccine; 4th International Veterinary Vaccines and Diagnostic Conference, Oslo, Norway *Epidemiology Infectious Diseases* 5(30): 5591 - 5597.

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

Domingo, M., Ferrer, L., Pumarola, M., Marco, A., Plana, J., Kennedy, S., McAliskey, M. and Rima, B. K. (1990). Morbillivirus in dolphins. *Nature* 38: 21 - 67.

Dundon, W. G., Kihu, S. M., Gitao, G. C., Bebora, L. C., John, N. M., Oyugi, J. O. and Diallo, A. (2017). Detection and Genome Analysis of a Lineage III Peste Des Petits Ruminants Virus in Kenya in 2011. *Transboundary and Emerging Diseases* 64(2): 644 – 650.

Emeli, T., Tebogo, K., Mikael, B., Niwael, M., Malamsha, E., Swai, J., Johasson, W. and Gerald, M. (2016). History and current status of peste des Petitis Ruminant Virus in Tanzania. Infectious Ecology and Epidemiology. *The One One Health Journal* 45(2): 34 - 56.

FAO and OIE (2015). Global strategy for the control and eradication of PPR. International conference for the control and eradication of Peste des Petits Ruminants (PPR).

Abidjan, Ivory Coast. [http://www.fao.org/3/a-i4460e.pdf]. Site visited on 2/5/2019.

FAO (2012). Peste des petits ruminants (PPR) situation in Africa. In: 7th Meeting of the GF-TADs Regional Steering Committee for Africa (SC7), Nairobi, Kenya. 16-17 July, 2012.

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

Gargadennec, R.G. and Lalanne, A. (1942). La peste des petits ruminants. Bulletin des Services Zoo techniques et des Epizooties de *l'Afrique Occidentale Française* 5: 16.

George, A., Dhar, P., Sreenivasa, B.P., Singh, R.P. and Bandyopahyay, S.K. (2006). The M and N genes based simplex and multiplex PCRs are better than the F or H gene based simplex PCR for peste des petits ruminants virus. Acta. *Virology* 50: 217 – 222.

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

Jones, P. G. and Thornton, P. K. (2009). Croppers to livestock keepers: livelihood ransitions to 2050 in Africa due to climate change. *Environmental Sciences and Policy* 12: 427 - 437.

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

Khalafalla, A., Saeed, I., Ali, Y.H, Abdurrahman, M.B., Kwiatek, O., Libeau, G., Obeida, A. and Abbas, Z. (2010). An outbreak of peste des petits ruminants (PPR) in camels in the Sudan. *Acta Tropical* 116 (2): 161-5.

Kumar, C.S., Raj, G. D., Thangavelu, A. and Shaila, M. S. (2007). Performance of RT-PCR-ELISA for the detection of peste des petits ruminants virus. *Small Ruminant research* 72 (2): 200-208.

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

Kwiatek, O., Ali, Y. H., Saeed, I.K., Khalafalla, A.I., Mohamed, O.I. and Obeida, A.A. (2011). Asian lineage of peste des petits ruminants virus, Africa. *Emergence Infectious Disease* 17: 1223 – 1231.

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

Kwiatek, O., Ali, Y. H., Saeed, I. K., Khalafalla, A. I., Mohamed, O. I., Abu Obeida, A., Abdelrahman, M. B., Osman, H. M., Taha, K. M., Abbas, Z., El Harrak, M., Lhor, Y., Diallo, A., Lancelot, R., Albina, E. and Libeau, G. (2011). *'Asian Lineage of Peste des Petits Ruminants Virus, Africa', Emerging Infectious Diseases* 17(7): 1223-1231.

Lefevre, P.C. and Diallo, A. (1990). Peste des petits ruminants. *Revue scientifique et technique Office international Epizootics* 9: 951–965.

Li, L., Bao, J., Wu, X., Wang, Z., Wang, J., Gong, M., Liu, C. and Li, J. (2010). Rapid detection of peste des petits ruminants virus by a reverse transcription loop-mediated isothermal amplification assay. *Journal of Virology Methods* 170 (1–2): 37– 41.

Libeau, G., Diallo, A., Colas, F. and Guerre, L. (1994). Rapid differential diagnosis of Rinderpest and Peste des Petits Ruminants using an immunocapture ELISA. *Veterinary Recearch* 134: 300–304.

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

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

Muhammad, M. (2014). Role of Wild small ruminants in the epidemiology of paste des petits. *Transboundary and Emerging Diseases* 61(5): 45 – 89.

Muhammad, M., Siamak, Z. and Mikael, B. (2013). Pathophysiology and Clinical Assessment of Peste des Petit Ruminants. *Molecular biology and Pathogenesis of Peste des Petits Ruminant Virus* 6: 33 – 48.

Munir, M. (2011). Diagnosis of Peste des Petits Ruminants under limited resource setting: A cost effective strategy for developing countries where PPRV is endemic. 1st. ed: VDM Verlag Dr. Müller 460pp.

Murphy, S.K. and Parks, G.D. (1999). RNA replication for the paramyxovirus simian virus 5 requires an internal repeated (CGNNNN) sequence motif. *Journal of Virology* 73: 805-809.

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

Norrby, E. and Oxman, M. N. (1990). Measles virus. *In Virology*, 2nd edition, I, Raven Press, New York. Pp. 1013 - 1044.

Ozkul, A., Akca, Y., Alkan, F., Barrett, T., Karaoglu, T., Daglap, S.B., Anderson, J, Yesilbag, K., Cokcaliskan, C., Genacy, A. and Burgu, I. (2002). Prevalence, distribution and host range of peste des petits ruminants virus, Turkey. *Emergence Infectious Diseases* 8: 708 -712.

Parida, S., Muniraju, M., Altan, E., Baazizi, R., Raj, G. D. and Mahapatra, M. (2016) 'Emergence of PPR and its threat to Europe', *Small Ruminant Research* 142: 16–21.

Pope, R. A., Parida, S., Bailey, D., Brownlie, J., Barrett, T. and Banyard, A. C. (2013). Early events following experimental infection with peste des petitsruminants virus suggest immune cell targeting. *Public Library of Science* 8 (2): 755 - 830.

Rahman, M.A., Shadmin, I., Noor, M., Parvin, R.E.H., Chowdhury, E.H. and Islam, M.R. (2011). Peste des petits ruminants virus infection of goats in Bangladesh: Pathological investigation, molecular detection and isolation of the virus. *Bangladesh Veterinary Journal* 28(1): 1 – 7.

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

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

Rossiter, P.B., Jessett, D.M. and Taylor, W.P. (1985). Microneutralisation systems for use with different strains of peste des petits ruminants virus and rinder pest virus. *Tropical Animal Health Production* 17(2): 75–81.

SADC (February 2012). SADC control strategy for pest de pestes ruminants. Southern African Development Community, Gaborone, Botswana. [http://www.rrafrica .oie.int/docspdf /en/2012/SADC_PPR_STRATEGY.pdf]. Site visited on 2/4/2019.

Saliki J.T., Libeau, G., House, J.A., Mebus, C.A. and Dubovi, E.J. (1993). Monoclonal antibody-based blocking enzymelinked immunosorbent assay for specific detection and titration of peste-des-petits ruminants virus antibody in caprine and ovine sera. *Journal of Clinical Microbiology* 31: 1075–1082.

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

Saravanan, P., Sen, A., Balamurugan, V., Bandyopadhyay, S.K. and Singh, R.K. (2008). Rapid quality control of a live attenuated Peste des petits ruminants (PPR) vaccine by monoclonal antibody based sandwich ELISA. *Biological* 36: 1–6.

Saravanan, P., Singh, R.P., Balamurugan, V., Dhar, P., Sreenivasa, B.P., Muthuchelvan, D., Sen, A., Aleyas, Ag, Singh, R.K. and Bandyopadhyay, S.K. (2004). Development of a N gene-based PCR-ELISA for detection of Peste-des-petits-ruminants virus in clinical samples, *Acta Virology* 48(4): 249 – 255.

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

Shankar, H., Gupta, V.K. and Singh, N. (1998). Occurrence of pest des petitis ruminants like disease in small ruminants in Uttar Pradesh. *Indian Journal of Animal Science* 68: 38 - 40.

Singh, R.K. and Bandyopadhyay, S.K. (2004). Development of an N gene-based PCR-ELISA for detection of Pestedes-petits-ruminants virus in clinical samples. *Acta Virology* 48: 249–255.

Singh, R.P., Sreenivas, B.P., Dhar, P. and Bandyopadhyay, S.K. (2004). A sandwich-ELISA for the diagnosis of Peste des petits ruminants (PPR) infection in small ruminants using anti-nucleocapsid protein monoclonal antibody. *Architect Virology* 149: 2155–2170.

Srinivas, R.P. and Gopal, T. (1996). PPR: A new menace of sheep and goats. *Livestock Advisor* 21(1): 22 - 26.

Swai, S.E., Kapaga, A., Kivaria, F., Tinuga, D., Joshua, G. and Sanka, P. (2018). Prevalence and distribution of pestes des petits ruminants virus antibodies in various districts of Tanzania. *Veterinary Research Communities* 33: 927 – 936.

Taylor, W. P. (1979). Serological studies with the virus of peste des petits ruminants in Nigeria. *Research of Veterinary Science* 26: 236 - 242.

Truog, T., Boshraf, H., Embry-Hyatt, C., Nfon, C., Gerts, V. and Tikoo, S. (2014). Peste Des Petit Ruminants Virus tissue tropisms and Pathogenesis in Sheep and Goats following experimental Infection. *Plos One* 9(1): 67 – 98..

Ularamu, H.G., Owolodun, O.A., Woma, T.Y., Audu, B. J., Aaron, G.B., Chollom, S.C. and Shamaki, D. (2012). Molecular diagnosis of recent suspected outbreaks of peste des petits ruminants (PPR) in Yola, Adamawa State, Nigeria. *African Journal Biotechnolgy* 11(5): 1158 – 1162.

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

APPENDICES

Appendix 1: TANAPA Authorization to sample wildlife in Serengeti

TANZANIA NATIONAL PARKS

OFFICE OF THE DIRECTOR GENERAL

P.O.BOX 3134, ARUSHA - TANZANIA

Ref. No: TNP/HQ/C.10/13 Date: 27/03/2018

Director General,
Tanzania Wildlife Research Institute,
P.O. Box 661,
ARUSHA.

Att: Dr. Emmanuel C. Mmassy

RE: INTRODUCTORY LETTER FOR DR. DANIEL MDETELE

This is in response to your letter Ref. No. TWRI/PPRP/318/VOL.II/2013/16 dated 23rd March, 2018 regarding the subject above.

I am pleased to inform you that permission is hereby granted to the Tanzanian Research Scientist Dr. Daniel Mdetele to enter and work in Serengeti National Park from **15th March, 2018 to 14th March, 2019** to conduct a project titled, ***"Pathway to Pest des Petits Ruminant Virus Elimination – Methods for Complex Ecosystems"***.

The researcher is required to abide by all park rules and regulations and should report to the Chief Park Warden of Serengeti to brief him before engaging in the research activities in the park.

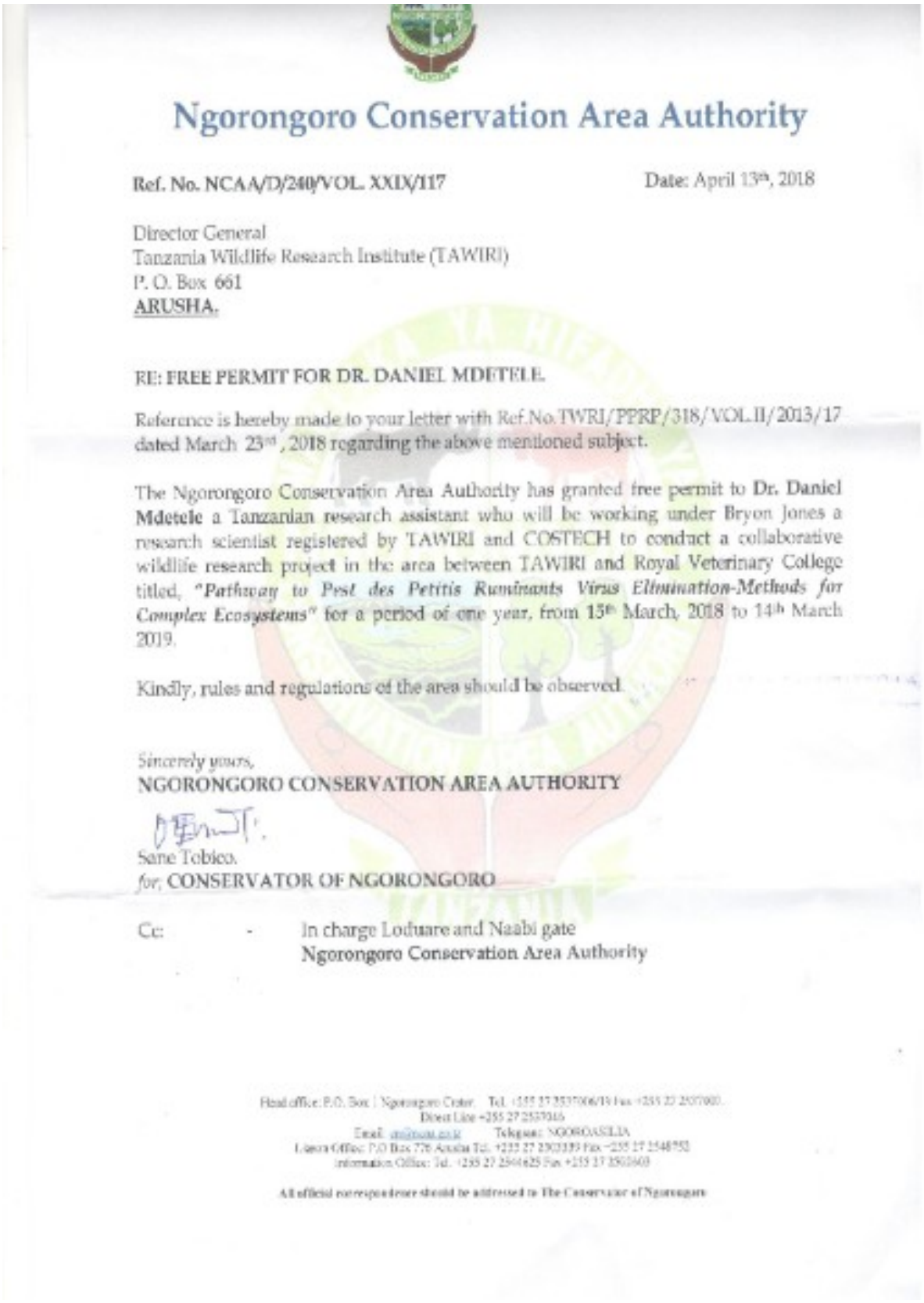
All national park rules and regulations apply.

Yours sincerely,
TANZANIA NATIONAL PARKS.

W. B. Kiwango
For: **DIRECTOR GENERAL**

Copy: Chief Park Warden – Serengeti.

Appendix 2: NCAA authorization to sample wildlife in Ngorongoro conserved area



Ngorongoro Conservation Area Authority

Ref. No. NCAA/D/240/VOL. XXIX/117 Date: April 13th, 2018

Director General
Tanzania Wildlife Research Institute (TAWIRI)
P. O. Box 661
ARUSHA.

RE: FREE PERMIT FOR DR. DANIEL MDETELE.

Reference is hereby made to your letter with Ref.No.TWRI/PPRP/318/VOL.II/2013/17 dated March 23rd, 2018 regarding the above mentioned subject.

The Ngorongoro Conservation Area Authority has granted free permit to **Dr. Daniel Mdetele** a Tanzanian research assistant who will be working under Bryon Jones a research scientist registered by TAWIRI and COSTECH to conduct a collaborative wildlife research project in the area between TAWIRI and Royal Veterinary College titled, *"Pathway to Pest des Petitis Ruminants Virus Elimination-Methods for Complex Ecosystems"* for a period of one year, from 15th March, 2018 to 14th March 2019.

Kindly, rules and regulations of the area should be observed.

Sincerely yours,
NGORONGORO CONSERVATION AREA AUTHORITY

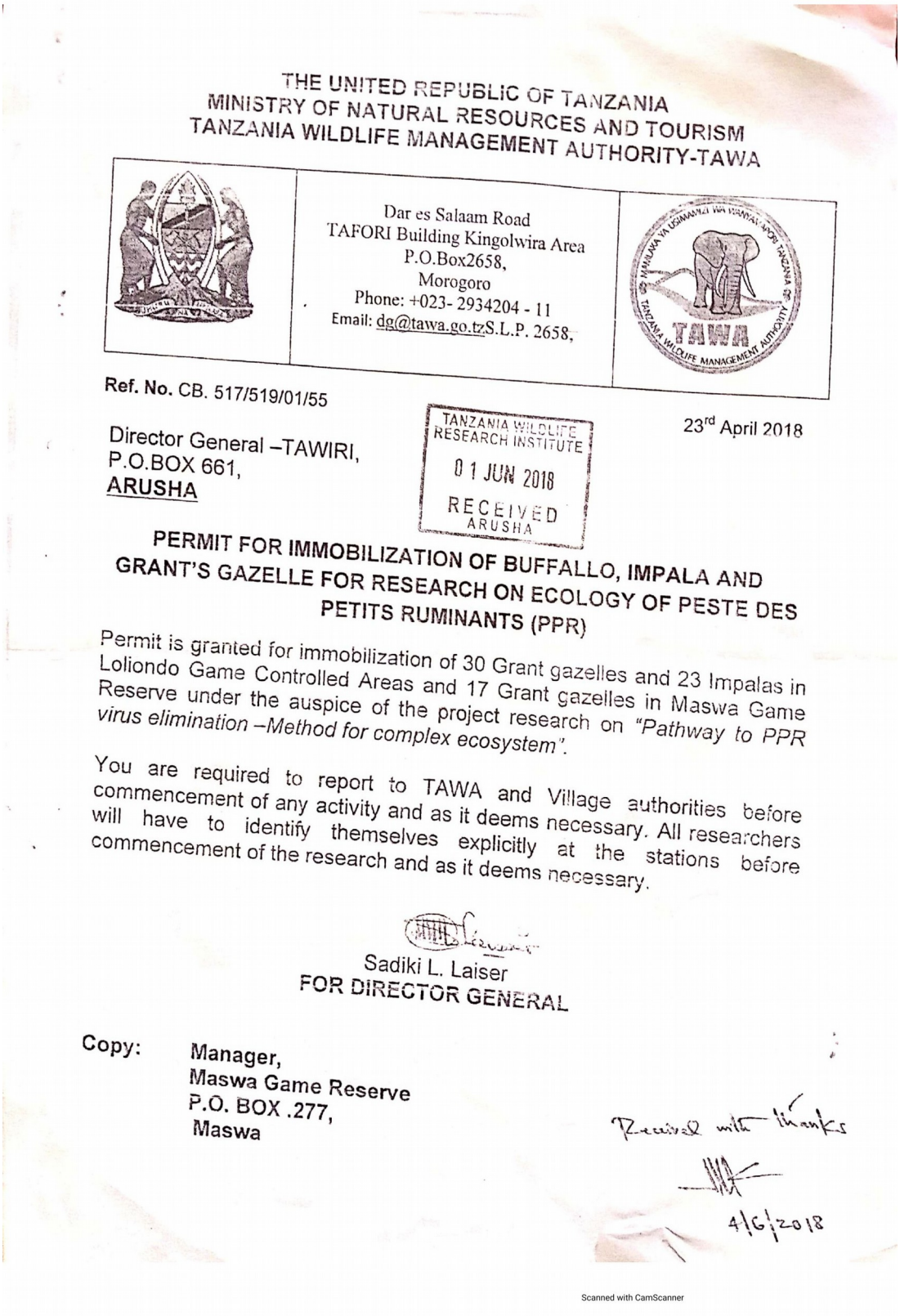
Sane Tobico.
for: **CONSERVATOR OF NGORONGORO**

Cc: - In charge Loduare and Naabi gate
Ngorongoro Conservation Area Authority

Head office, P.O. Box 1 Ngorongoro Crater. Tel. +255 27 2537006/19 Fax +255 27 2537000
Direct Line +255 27 2537015
Email: ... Telegram: NGOROASELIA
Liaison Office: P.O Box 776 Arusha Tel. +255 27 2502339 Fax +255 27 2548752
Information Office: Tel. +255 27 2564625 Fax +255 27 2502603

All official correspondence should be addressed to The Conservator of Ngorongoro

Appendix 3: TAWA authorization to sample wildlife in Loliondo game controlled area



THE UNITED REPUBLIC OF TANZANIA
MINISTRY OF NATURAL RESOURCES AND TOURISM
TANZANIA WILDLIFE MANAGEMENT AUTHORITY-TAWA

Dar es Salaam Road
TAFORI Building Kingolwira Area
P.O.Box2658,
Morogoro
Phone: +023- 2934204 - 11
Email: dg@tawa.go.tzS.L.P. 2658,

TAWA
TANZANIA WILDLIFE MANAGEMENT AUTHORITY

Ref. No. CB. 517/519/01/55

23rd April 2018

Director General –TAWIRI,
P.O.BOX 661,
ARUSHA

TANZANIA WILDLIFE RESEARCH INSTITUTE
01 JUN 2018
RECEIVED
ARUSHA

PERMIT FOR IMMOBILIZATION OF BUFFALLO, IMPALA AND GRANT'S GAZELLE FOR RESEARCH ON ECOLOGY OF PESTE DES PETITS RUMINANTS (PPR)

Permit is granted for immobilization of 30 Grant gazelles and 23 Impalas in Loliondo Game Controlled Areas and 17 Grant gazelles in Maswa Game Reserve under the auspice of the project research on *"Pathway to PPR virus elimination –Method for complex ecosystem"*.

You are required to report to TAWA and Village authorities before commencement of any activity and as it deems necessary. All researchers will have to identify themselves explicitly at the stations before commencement of the research and as it deems necessary.

Sadiki L. Laiser
FOR DIRECTOR GENERAL

Copy: **Manager,**
Maswa Game Reserve
P.O. BOX .277,
Maswa

Received with thanks
4/6/2018

Scanned with CamScanner

Appendix 4: House hold interview report form

Interview code		Date	

Name(s) of main interviewee(s)					
Village		Ward		District	
Interview type	Individual / group		No. interviewees	Male	Female

1. Introduction: introduce the project, explaining that you are collecting information about the sheep and goat production system in this area the disease problems that are faced.

Ask if they are willing to answer a few questions - participation is voluntary.

(Depending on time available and level of interest of the household members, try to cover some or all of the topics)

2. Sheep and goat diseases: we would like to know about the sheep and goat diseases that are troubling you in this area?

- List the diseases with local name
- Ask the characteristics of each disease, focussing more on the PPR-like diseases (respiratory, diarrhoea, fever, discharges);
    - the main clinical signs
    - the cause
    - Which species and ages are affected?
    - Which season?
    - How do you treat or prevent the disease?
- Which of these diseases causes significant losses to you?

3. Disease prevention/control: what do you normally do to prevent diseases?

- Vaccinations, parasiticides, supplements, traditional methods?

4. Grazing patterns: where do you normally take your sheep and goats for grazing? And for water?

- What about during the dry season – grazing, water?
- And the rainy season?
- Who normally takes the sheep and goats for grazing?

5. Contact with other flocks: who do you meet in the grazing area?

- In the dry season
- In the wet season?

6. Wild animals: do you meet wild animals in the grazing land, or at the watering points?

- What species?
- How close do the herbivores come to the sheep and goats?
- Have you seen any sick or dead wild herbivores in the past one year?

7. What do you do to increase the size of the flock?

- Where do you buy sheep or goats?
- Other reasons for animals joining the flock?
- What time or times of year do the sheep normally give birth?
- And when do the goats give birth?

8. Do you ever sell any sheep or goats?

- Where do you sell them?

9. Are all your sheep and goats here in this flock?

- Do you ever give or loan sheep or goats to other people, or receive any from other people? Can you give some examples of these from the past one year (to/from who, to/from which place, reason – marriage, assistance, etc.)

10. Livelihoods: Apart from sheep and goats, what other activities are you doing for income?

11. Closing – thank you, give advice, do they have any questions for you?

12. Clinical cases: finally – do you have any sick sheep or goats at the moment that we can look at?

Flock observation – if the flock is available:

- How is the flock enclosed at night, during daytime?
- estimate the flock size through observation
- What proportion are sheep/goats?
- Any clinical cases? Record the main clinical signs, types of animals affected the local name for the disease problem.

Appendix 5: Outbreak Investigation report form

Owner name		Main informants	
Village		Date of investigation	
Ward		GPS Coordinates: latitude	
District		GPS Coordinates: longitude	

1. Date of onset of first case	

2. Animals affected	Goats	Sheep	Remarks (age groups, sex, breed)
No. sick			
No. dead			
Total no. in flock			

3. Clinical signs	Goats	Sheep
Clinical signs		
No. abortions		
Effect on milk production?		
Effect on body condition/growth?		
Effect on marketing?		
Post mortem signs		

4. What local name do you call this disease? - What does this name mean?	

5a. Have you seen this disease before in your flock?	
5b. When?	

6. Are any other flocks in the area affected? - number of flocks and their location - Any contact with this flock?	

7a. What treatment or control measures have you applied?	
7b. What effect did you observe?	

8a. Has the flock been vaccinated against PPR?		8b. When?	
8c. What other diseases has the flock been vaccinated against?		8d. When?	

9. What other disease problems have you seen in the flock in the past one year? - local names, meaning - clinical signs	

10. Does your flock have contact with other flocks?	

- When and where?	

11a. How long has your flock been in this location?	
11b. If the flock moved in the past year, when, where and why was it moved?	

12a. Did you buy any sheep or goats in the past one month?	
12b. Did you take any sheep or goats to the market that were unsold and returned to the flock in the past month?	

13. Has the flock been in contact with any wild animals? - Which type of wild animals? - When and where? (grazing, water point, other) - Type of contact?	

14. Have you seen sick or dead wild animals? - Species? - When and where? - Clinical signs?	

15. Any other remarks	

Appendix 6: Clinical examination form for sick animals

Owner name: **Village:** **Ward:** **District:**
Code:

No.	Species		Sex		Dentition (pairs perm incisors)					Age	Temp ^{0}C	Ocular signs; discharge, other	Nasal discharge	Oro-nasal lesions	Respiratory signs, rate	Faeces	PPR rapid test result	Remarks
	G	Sh	M	F	0	1	2	3	4									

Notes: 1. Ocular discharge/other: watery, mucoid, purulent, redness, congestion, opacity. 2. Nasal discharge/other: watery, mucoid, purulent, lesions. 3. Oral lesions/other: congestion, lesion type, limited/widespread, salivation. 4. Respiratory signs/rate: tachypnoea (normal goat 10-30/min, sheep 15-40/min), cough, dyspnoea. 5. Faeces: firm/soft/runny, haemorrhage, mucus. 6. Remarks: general condition, activity, depression, appetite, able to stand.

File S1 – Key informant interviewes

Appendix 7: Clinical examination form for sick animals

District	Livestock production system	Ethnique groups	Wildlife livestock interaction	Livestock movements	Livestock population	Administrative structure and animal health personnel	Common diseases and	Animal health facilities and services	PPR Vaccination	Livestock marketing
Karatu	Pastoralists and agropastoralists	Iraq (Mbulu) - agropastoralist, Barabaig (Mangati) and Maasai – pure pastoralists. Hadzabe found around lake Eyasi mostly hunter and gatherers.	At Lake Eyasi escarpment animals move up and down to Ngorongoro. During the dry season, people (Iraq and Barabaig) take their animals into the forest at night,	move animals to Shinyanga, Singida, Tanga, Dodoma, Coastal		The district has four divisions; Mburumburu, Karatu, Endabashi and Mang'ola, 4 wards and 62 villages.	Ormilo, CCPP , Helminthosis and Brucellosis	The two vehicles are grounded. Some LFOs have motorbikes. They have a functional fridge-freezer	The last PPR vaccination was in 2008-9 under the VACNADA project. In 2015-16 Alphavet was conducting combined PPR and pox vaccination in Ngorongoro and neighbouring areas (Morocco vaccine)	Karatu, Mang'ola, Endabash, Mbulumbulu , Matala, Basodawish.
Longido	95% Pastoralist	about 90% Maasai	Wildlife share grazing and water with livestock, there are wildlife in all wards; antelopes,	Movement is to Monduli, Kilindi, Siaya (Moshi), Manyara, Kenya	Livestock population: 215,000 cattle, 400,000 goats	The district has 4 divisions, 18 wards and 48 villages. There is one DVO, 3 Livestock Officers, 17	Ormilo, CCPP, PPR, Sheep and goat pox	Three fridge/freezers , large/medium and small, but only one large cool box and one small one.	Last PPR vaccination in 2012 by VACNADA Project. They have a vaccination calendar, and	Longido, Kitumbeine, Noondoto, GelaiLumbwa, GelaiMeirugoi, Mairowa (EngareNaib

			giraffe, wildebeest, especially in Tingatinga (east), Gelailumeigoi (west) and Ketumbeine (east).		and 300,000 sheep	Livestock Field Officers and 1 rangeland officer	,orf	LFOs have own cool boxes	PPR is scheduled for April because they tend to see PPR in June-July.	or), Ilkaswa, Ngeriani, Sinya, Kamwanga, Engekaret, MIVARF near Namanga.
Meatu	Agro pastoralists	Sukuma; transhumant pastoralists keeping cattle, sheep and goats, Mang'ati; like Taturu they don't move far, but may move to NCAA	Maswa Game Reserve – livestock are not allowed to enter, but people take them in; cattle , sheep and goats Makao WMA animals allowed grazing within the WMA during harsh times but not during the rainy season.			There 2 divisions, 29 wards and 109 villages. Staff; 1 DVO, 5 Livestock Officers, 27 LFOs (9 in the HQ and 26 in the field, 22 diploma holders, 2 certificates, 3 laboratory officers – but no lab) and 2 rangeland officers.	CCPP, babesiosis and other tick-borne diseases, coccidiosis and coenurosis.		The last PPR vaccination conducted in the district was in 2014, and there was no private vaccination being carried out	Bukundi, Sangaitinje, Malwiro, Mwanhuzi, Usiulize – Animals are taken to Mwanza, Arusha, Dar es Salaam, and to Kenya via Msoma and Tarime.
Monduli	mostly pastoralist, with	Maasai and its group	About 75% of the district is multiple land	Animals seasonally migrated	According to the 2012	Three divisions; Kisongo,	Contagious Caprine PleuralPneu	Cold chain: there were two fridges in	The last PPR vaccination by the government	Kigongoni Engaruka

	some agro-pastoralists,		use with humans, livestock and wildlife. Southern part of district is wildlife corridor.	between Monduli, Longido, Ngorongoro, Karatu, Babati, Simanjiro and Arumeru	census there were 162,188 sheep and 186,266 goats in the district	Makuyuni and Manyara. There 20 wards and 61 villages. Animal health personnel: one DVO, 5 Livestock Officers, 28 Livestock Field Officers	monia, Coenurosis, Sheep and goat pox, Orf and Diarrhoea	Monduli town (Kisongo Division) and a freezer for ice packs, and one fridge in each of the other two divisions.	had been in 2013. Farmers wanting vaccination ask the LFO who purchase vaccine from the private vet shops vaccinate for pay.	Selela Makuyuni Nanja – Meserani Monduli juu Monduli Mjuini Mbuyuni Loksale There are also informal markets.
Ngoron goro	Pastoralists	100% Maasai	Seasonally animals migrate into the Loliondo Game Controlled area; Masherin, Olchoro Oybor and Alamana, which are close to Serengeti National Park.	Graze between, Kenya and Tanzania	cattle 505,657, 791,270 goats and 856,646 sheep determined during 2012	Animal health personnel are 1 DVO, 4 Livestock Officers, and 16 Livestock Field Officers. Some wards have no LFO;	CCPP, coenurosis, anthrax	Deep freezer and three working fridges, one large cool box and 3 small ones, and a vehicle fridge, as well as deep freezers in Samunge and Arash. Also, borrow equipment from the	LFOs buy vaccine from Arusha; people come with vaccine from Kenya and vaccinate people’s herds.	Ololosokwan, Soitsambu, Arash, Piyaya, Malambo, Pinyinyi, Engerasero, Olbalbal, Nainokanoka, Alaytole, Olmesuti, Esere, Endulen, Kakesio, Osinoni,

								health department. Transport: there is one car and 9 motorcycles of which 5 are working,		Alailelai, Oldonyosambu, Naiyobi and Wasso
Serengeti	Agro-pastoralists, cultivating crops and keeping cattle sheep and goats.	approximately half Kuria, Koma and Ngoreme (different sub-groups of Kuria)	Approximately half of Serengeti NP is within Serengeti District, and most of the wildlife in the park, close to the settlement areas		sheep population is greater than goats	There are 4 divisions, 30 wards, 78 villages, as well as some sub-villages. The animal health personnel are; 1 DVO, 4 Livestock Officers, 18 LFOs, 1 range officer, 1 extension officer and 2 animal scientists.	Sheep and goat pox Tick-borne disease – anaplasmosis is rampant Orf CCPP	Two fridges (one not working), 1 deep freezer, 2 vehicle fridges, 3 big vaccine carriers. one working vehicle and one not working	There was PPR vaccination in 2012 supported by VACNADA, but none since and there is no private use of vaccine	Mugumu Lung'abule, Masinki, and Isenye, Majimoto, Kisaka, Nyasurura and Musati. Informal markets; Machochwe, Mbalimbali, Nyambuli, Melenga, Monuna, and Omaki

Appendix 8: File S1 – Key informant interviewes

	3. Clinical signs												4. Local disease name		5. Seen disease before		6. Other flocks affected		
OBI code	Goats clinical signs	Goats no abortion	Goats effect on milk	Goats effect on body condition	Goats effect on market	Goats post mortem signs	Sheep clinical signs	Sheep no. abortion	Sheep effect on milk	Sheep effect on body condition	Sheep effect on marketi	Sheep post mortem signs	Local disease name	Meaning of name	Seen before in flock?	When seen before?	Any other flocks affecte	Number of affected flocks	Any contact with this flock?
NGR1	Difficult breathing, diarrhoea and lacrimation	no	no - affect	recovered	NA	NA	difficult breathing, diarrhoea and lacrimation	no	no - affect	recovered	NA	NA	don't know	NA	yes	2 years ago	no respon	(there are	NA
NGR2	no	no	no	emaciation	NA	NA	Nodular lesion - looks like healing pox lesions	no	no - affect	emaciatio	NA	NA	Olomoroji	Nodular lesions	Yes	1 year ago	NA	NA	NA
NGR3	diarrhoea	no	no	yes	NA	Haemorrhagic lung	none	no	no	no	NA	NA	Engorotik	diarrhoea	Yes	seeing case	No	none	NA
KR1	conjuctivitis, lacrimation, mouth ulcers and orf-like lesion on lips, slight nasal discharge	1	no	no	no	no	none	no	no	no	no	no	Salapta	mouth lesion	yes	1 year ago	No	none	none
NGR4	diarrhoea, pale mucous membrane and rough hair, weak and emaciated	6	no milk	highly ema	yes	lung affected	swelling of neck	NA	NA	NA	NA	NA	Orkipei	lung disease	yes	every year	yes	NA	yes
NGR5	none	no	no	no	no	no	conjuvtival inflamation, lacrimation and diarrhoea	1	no	no	no	NA	Olodwa	enlarged gall bladder	yes	last week	yes	NA	yes
NGR6	no	no	no	no	no	no	lacrimation, nasal discharge, diarrhoea	no	reduced	no	NA	enlarged l	olodwa/enkorotiK	diarrhoea	yes	during rainy	yes	NA	NA
NGR7	conjunctivitis, diarrhoea, oral ulceration, ocular discharge, purulent nasal discharge, rough hair coat	1	low milk p	emaciation	no	ulceration of mouth	diarrhoea and ulcerations	no	no	no	no	NA	Orkipei	lung disease	yes	Apr-18	no	10	yes
NGR8	diarrhoea and emaciation	no	reduced	emaciated	can't take t	oedematous meat	diarrhoea, emaciation	no	not milked	emaciatec	cannot take	dead tape	Entonyaa	diarrhoea	yes	past 3 mont	yes	almost ev	yes
NCAA1	diarrhoea, coughing and nasal discharge	2	no	emaciation	no	Fluid in abdominal	diarrhoea, coughing and nasal discharge	no	NA	emaciatio	no	NA	ordwaa	enlarged bile	NA	NA	yes	NA	during gr