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Epidemiology, risk factors and diagnostic accuracy of surveillance methods of selected diseases presenting with respiratory signs in small ruminants in Tanzania

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EPIDEMIOLOGY, RISK FACTORS AND DIAGNOSTIC ACCURACY OF SURVEILLANCE METHODS OF SELECTED DISEASES PRESENTING WITH RESPIRATORY SIGNS IN SMALL RUMINANTS IN TANZANIA

Andrew Claud Chota

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology Arusha, Tanzania

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ABSTRACT

Outbreaks of diseases presenting with respiratory signs in small ruminants in Tanzania have been reported for years. Concurrent infections complicate diagnosis resulting in untimely interventions and significant economic losses. A cross-sectional survey was conducted between June, 2016 and July, 2017 to determine the risk factors associated with small ruminants exposure to *Mycoplasma capricolum* subsp. *capripneumoniae* (*M. capripneumoniae*) and small ruminant morbillivirus (SRMV). Outbreak investigations were conducted between June, 2016 and July, 2018 to validate the performance of the existing and improved disease surveillance systems and to evaluate the accuracy of the reports, the data were also used to determine the level of concurrent infections in those outbreaks. Risk factors data were analysed using logistic regression models. The field data forms (FD-1 and FD-2) performance were analysed using inter-rater reliability test against the gold standard test. The percentage proportions were used to describe levels of concurrent infections. Risk factors for *M. capripneumoniae* occurrence in goats were farming system (OR = 0.91073332), mixing species (OR= 1.0793679) and grazing with wild animals (OR=1.0546803). Occurrence of SRMV in goats was influenced by farming system (OR=1.154254), mixing of flocks (OR=1.060278) and grazing with wild animals (OR=1.077164). Risk factors for occurrence of *M. capripneumoniae* in sheep were farming system (OR=0.9310771) and presence of PPR (OR=1.0564274) whereas, SRMV was influenced by farming system (OR=1.188681), mixing flocks (OR=1.135146) and previous diseases outbreak (OR=1.161883). In outbreak investigations, FD-2 performed better than FD-1 in the diagnosis of both CCPP (PPV = 22.2% Vs 16.2%) and PPR (PPV = 50.0% Vs 43.2%) in goats and in sheep, in the diagnosis of PPR (PPV = 26.3% Vs 23.1%). In determining concurrent infections, 79.1% (117/148) of the goats and 28.1% (16/57) of the sheep had concurrent infections. The SRMV and *Pasteurella multocida* (*P. multocida*) in goats and SRMV in sheep were the pathogens involved in co-infections. This study reports several risk factors being associated with exposure of small ruminants to *M. capripneumoniae* and SRMV and that, the current disease surveillance system does not provide reliable accurate data on outbreaks with co-infections in which *P. multocida* are involved and should be considered in control strategies.

DECLARATION

I, Andrew Claud Chota, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology (NM-AIST) that this thesis is my original work and that it has neither been nor being concurrently submitted for consideration of a similar degree award in any other institution.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend the thesis entitled "*Epidemiology, risk factors and diagnostic accuracy of surveillance methods of selected diseases presenting with respiratory signs in small ruminants in Tanzania*" submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Life Sciences at Nelson Mandela African Institution of Science and Technology.

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DEDICATION

This PhD work is dedicated to my parents, the late Mr. Claud Paul Chota (May his soul rest in eternal peace) and Mrs. Rosina Joseph Mponzi for their encouragements all through my academic career, also to my wife and children to inspire them reach their goals and dreams.

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LIST OF ABBREVIATIONS AND SYMBOLS

$	United States Dollar
%	Percentage
®	Registered
µl	Micro litre
^{0}C	Degrees of Centigrade
™	Trade Mark
κ	Kappa
Bov.	Bovine
bp	Base pair
BST	Bone marrow Stroma
CAP	Catabolite Activator Protein
CBPP	Contagious Bovine Pleuropneumonia
CCPP	Contagious Caprine Pleuropneumonia
CCẍ	Conjugate Control Mean Absorbance
cELISA	Competitive Enzyme Linked Immunosorbent Assay
CFT	Compliment Fixation Test
Coef.	Coefficient
Dr	Doctor
DVO	Distric Veterinary Officer
EDTA	Ethylene Diamine Tetraacetic Acid
ELISA	Enzymes Linked Immunosorbent Assay
FAO	Food and Agricultural Organization of the United Nations
FD-1	Field Data Forms-1
FD-2	Field Data Forms 2
Fig	Figure
gDNA	Genomic Deoxyribose Nucleic Acids
HH	Households
IAEA	International Atomic Energy Agency
ID no	Identification number
ID	Innovative Diagnostics
kb	Kilo base
Lab	Laboratory

LD	Laboratory Data
LFO	Livestock Field Officer
LiSBE	Life Science and Bio-Engineering
LppA	Lipoprotein A.
LSNR	Livestock Sector National Report
Mab	Monoclonal antibodies
MabCẍ	Mab Control Mean Absorbance
MLFD	Ministry of Livestock and Fisheries Development
Mond.	Monduli
NC	Negative Control
NM-AIST	Nelson Mandela Institution of Science and Technology
nov.	novel
NR	Not Reported
OD	Optical Density
OIE	Office Internationale des Epizooties
OMP	Outer Membrane Protein
OR	Odds ratio
PC	Positive Control
PCR	Polymerase Chain Reaction
PEHPL	Program for Enhancing the Health and Productivity of Livestock
PhD	Doctor of Philosophy
PM	Pneumonic mannheimiosis
PP	Pneumonic pasteurellosis
PPR	Peste des petits ruminants
PPRV	Peste des petits ruminants virus
PPV	Positive predictive value
REA	Restriction Enzymes Analysis
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribose Nucleic Acids
rRNA	Ribosomal RNA
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SA	Sample Absorbance
sp	Species
SPI	Sample Percentage Inhibition

SRMV	Small Ruminant Morbillivirus
SSA	Serotype Specific Antigen
subsp	sub species
URT	United Republic of Tanzania
USA	United States of America
USD	United States Dollar
v/v	Volume by volume
VNT	Virus Neutralization Test
ZVC	Zonal Veterinary Centre

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Small ruminants (sheep and goats) play an important role as source of income and protein to over 330 million resource constrained families in Africa and Asia (Office Internationale des Epizooties [OIE] & Food and Agricultural Organization [FAO], 2015). Africa and Asia comprise of, over 80% of the World's 1.7 billion sheep and goats (OIE & FAO, 2015). Tanzania has the third largest livestock population in the African continent comprising 25 million cattle complimented by 16.7 million goats, 8 million sheep, 2.4 million pigs and 36 million chickens (United Republic of Tanzania [URT], 2015). Small ruminants are the most widely distributed species and adapted to different agro-ecological zones (Livestock Sector National Report [LSNR], 2012). Goats are hardy and well adapted to harsh environment and can browse on plants that are not eaten by other livestock. Therefore, keeping goats and other grazing livestock makes more efficient use of the natural resource base and adds flexibility to the management of livestock (Silanikove, 2000). Furthermore, small ruminants play a triple role as a means of subsistence, store of wealth and source of income (Mwambene *et al*., 2014). This shows the importance of small ruminants to the resource constrained communities.

Despite the importance of small ruminants to over 75% of the one billion people living under less than two USD per day in Africa and Asia (OIE & FAO, 2015), their production potentials have not been attained. For instance, the average slaughter weight of local breeds of animals of Africa and Asia is much lower as compared to exotic breeds of North America and Europe. Flock size, genetic improvement and diseases control are lagging far behind in Africa as compared to developed countries (Devendra, 1999). In Tanzania, lack of fully utilization of available land and water, lack of knowledge on management, low genetic potential, inadequate exploitation of the potentials of indigenous breeds and presence of diseases and in particular, transboundary diseases pose a great challenge to small ruminants production (Ministry of Livestock and Fisheries Development [MLFD], 2010).

Small ruminants succumb to a number of diseases that contribute to low productivity and hence, threatening the poor families' economic gains. Internal parasites associated with gastro-enteritis in small ruminants include *Haemonchus contortus, Ooesophagostomum*

columbianum and Trichostrongylus colubriformis. Lungworms include *Dictyocaulus filaria*, *Protostrongylus rufescens* and *Muellerius capillaris* (Bell, 2008; Zvinorova *et al*., 2016). Gastrointestinal parasites cause significant economic losses in small ruminants especially the young animals in wet seasons (Zvinorova *et al*., 2016). Sheep also succumb to viral diseases like Maedi-Visna, a respiratory-nervous system complex disease of sheep, ovine pulmonary adenocarcinoma and Nairobi sheep disease (Bell, 2008; Ritchie *et al*., 2012). Other viral diseases which affect small ruminants and were believed to be highly localized in Africa include PPR and Rift Valley fever (Blomström *et al*., 2016; Birindwa *et al.*, 2017). Bacterial diseases commonly inflicting small ruminants include pneumonic pasteurellosis and pneumonic mannheimiosis caused by *Pasteurella multocida* and *Mannheimia hemolytica,* respectively, the causative agents are normal commensals of the respiratory tract (Engdaw & Alemneh, 2015). Furthermore, mycoplasmas under the class *Mollicutes* (Jensen, 2017), affect small ruminants causing significant losses (Litamoi *et al*., 1990; Bölske *et al*., 1995; Kusiluka *et al*., 2000).

In most of the developing countries, domestic small ruminants are affected by diseases presenting with respiratory signs with multiple pathogens being implicated (Birindwa *et al*., 2017; Habashy *et al*., 2009; Hernandez *et al*., 2006; Kgotlele *et al*., 2014a). These include, Contagious caprine pleuropneumonia (CCPP) a disease of goats caused by *Mycoplasma capricolum* subsp. *capripneumoniae (M. capripneumoniae)* and other mycoplasma pneumonias caused by a number *of Mycoplasma* species (Thiaucourt *et al*., 1996); pasteurellosis caused by *P. multocida* and mannheimiosis caused by *M. haemolytica* (Marru *et al*., 2013) and peste des petits ruminants (PPR) a disease of domestic small ruminants and wild artiodactyls caused by the small ruminants morbillivirus (Khan *et al*., 2012; Fine *et al*., 2020). In Tanzania, CCPP has been reported in several locations including, Morogoro (Kusiluka *et al*., 2000), Kongwa and Mvomero (Shija *et al*., 2014) and Southern zone (Mbyuzi *et al*., 2015). Contagious caprine pleuropneumonia (CCPP) and PPR are transboundary diseases that spread fast across borders and over a large area. Peste des petits ruminants (PPR), which was initially believed to be a disease of Africa, Middle East and the Indian subcontinent has now extended to the Far East. Both CCPP and PPR cause high morbidity and mortality rates, with CCPP morbidity ranging from 80-100% and mortality 60-100% whereas; PPR can cause 100% mortality in naive animals and 20% in endemic areas (FAO, 2013). In Tanzania, PPR has been reported in small ruminats in Loliondo, a village in Serengeti district and southern zone in Mtwara (Muse *et al.,* 2012; Kgotlele *et al.,* 2018) and

serological evidence for SRMV in wild life was reported in Ngorongoro district close to Serengeti ecosystem (Mahapatra *et al.,* 2015).

Pneumonic pasteurellosis and pneumonic mannheimiosis have a worldwide distribution and their causative agents are normal commensals of the respiratory system and infection occurs when there is lowered immunity caused by other conditions such as infections, stress and extreme weather conditions (Mohamed & Abdelsalam, 2008). In Tanzania, presence of *Pasteurella* infections in small ruminants was reported for the first time in the central zone (Noah *et al.*, 2011).

Diagnosis of CCPP has always been a challenge mainly because of fastidious nature of the causative agent (*M. caprineumoniae*), making it difficult to isolate through culture. This could be the reason why the causative agent of the disease was described in Kenya in 1976 (MacOwan & Minette, 1976), over a century after the disease was described in Algeria in 1876 (Thiaucourt & Bölske, 1996). Contagious caprine pleuropneumonia (CCPP) was confirmed in Tanzania in 1998 (Msami *et al.*, 1998) and later isolated in 2000 (Kusiluka *et al.*, 2000). Advanced techniques like molecular identification and competitive Enzyme Linked Immunosorbent Assay (cELISA) with monoclonal antibodies (Mab) specific to *M. capripneumoniae* are used in diagnosis (Peyraud *et al.*, 2014; Ahmad *et al.*, 2020). As a control method for CCPP, vaccinations are advocated as it may provide protection of up to 100% (Giadinis *et al.*, 2008). However, adoption of the vaccines for CCPP and availability of the same for use in field is limited (Wambura *et al.*, 2014). Pneumonic pasteurellosis and pneumonic mannheimiosis can be diagnosed by isolation of their causative pathogens (Shiferaw *et al.*, 2004), use of molecular techniques (Legesse *et al.*, 2018; Rawat *et al.*, 2019) or serological techniques (Assefa *et al.*, 2018). Diagnosis of PPR requires serological techniques like virus neutralization test, virus specific monoclonal antibodies in immunocapture ELISA, isolation of the virus in Vero cells and use of molecular techniques (OIE, 2019). However, presumptive diagnosis for CCPP, other mycoplasmas pneumonia, pneumonic pasteurellosis and pneumonic mannheimiosis can be based on clinical signs (Ekong *et al.*, 2014; Hernandez *et al.*, 2015; Taunde *et al.*, 2019; Teshome *et al.*, 2019), postmortem and histological examinations like adhesive pleurisy, fibrinous bronchopneumonia and neutrophils infiltration in alveoli (Radi *et al.*, 2002; Mohammed & Abdelsalam, 2008; Teshome *et al.*, 2019). In the diagnosis of PPR, diarrhea, presence of vesicular lesions on the gums and buccal cavity are suggestive, zebra stripes at the ileocaecal

junction is pathognomonic (Islam *et al.*, 2014). Generally, molecular techniques, ELISA and even isolation for *M. capripneumonia* and SRMV are difficult to apply in rural settings because they require sophisticated equipment and expertise. Like in many countries, livestock populations in Tanzania are in rural areas.

Failure in controlling CCPP and PPR results into severe economic losses due to mortality and exclusion from international trade whereas pneumonic pasteurellosis and pneumonic mannheimiosis contribute significantly to aggravate to the losses. Contagious caprine pleuropneumonia (CCPP) causes an estimated loss of $507 million per annum in endemic areas (Global Allience for Livestock Veterinary Medicine [GAL*Vmed*], 2018). Peste des petits ruminants (PPR) in Tanzania causes approximately a loss of more than €92 million at national level per annum (OIE, FAO and International Atomic Energy Agency [IAEA], 2013), whereas, the same causes losses estimated to range between USD1.45 to USD 2.1 billion per annum worldwide (OIE & FAO, 2015). This study, expounded the epidemiological context of CCPP and PPR and the level of association of opportunistic secondary bacteria, the accuracy of the field reports which are used in making diagnostic and control policies, and establish the level of concurrent infections in the outbreaks of diseases which present with respiratory signs.

1.2 Statement of the problem

Small ruminants are affected by diseases presenting with respiratory signs, with reported outbreaks and mortality persisting for a long time (Kgotlele *et al.*, 2018). Major diseases that are associated with these outbreaks in Tanzania are CCPP and PPR. These are transboundary diseases which cause significant socio-economic losses in resource constrained families (OIE & FAO, 2015). Occurrence of these diseases is also associated with occurrence of pneumonic pasteurellosis and pneumonic mannheimiosis which are caused by respiratory opportunistic bacteria *P. multocida* and *M. haemolytica* (Mohamed & Abdelsalam, 2008) following physiological stress. Despite, the fact that these two diseases at one point show similar clinical signs and postmortem lesions (Abd El-Rahim *et al.*, 2010; Khan *et al.*, 2012; Muse *et al.*, 2012; Kgotlele *et al.*, 2014b; Birindwa *et al.*, 2017; Rahman *et al.*, 2018), they have not been addressed together, so co-infections are not well established world-wide. Furthermore, the clinical signs and postmortem lesions though similar, have not been closely examined to associate with co-infections dynamics. In many developing countries including Tanzania, laboratory facilities are limited, especially in rural areas where most of the animals are reared.

This makes it difficult for veterinarians to rely on laboratory facilities for diagnosis and hence, they rely on clinical signs and postmortem. These diagnoses form an important source of information for policy making by national veterinary authorities while the accuracy of such data is not known and is compounded by misdiagnosis (Halliday *et al.*, 2012). Establishing the distribution of the diseases, knowledge on concurrent infections and detailed documentation of clinical and postmortem lesions will improve the diagnosis and hence the accuracy of the reports used to inform policy and improve management. Therefore, the gathered information will help in improving policy, interventions and in putting appropriate control strategies.

1.3 Rationale of the study

Diseases presenting with respiratory signs cause significant losses in families that relie on small ruminants for their livelihood. Contagious caprine pleuropneumonia (CCPP) causes an estimated loss of $507 million per annum in endemic areas (GAL*Vmed*; 2018). Peste des petits ruminants (PPR) in Tanzania causes approximately a loss of more than €92 million at national level per annum (OIE, FAO & IEAE, 2013), whereas, the same causes losses estimated to range between USD1.45 to USD 2.1 billion per annum worldwide (OIE & FAO, 2015).

Risk factors for the spread of the two major transboundary diseases have for quite a long time been studies separately. However, these two diseases at one point show similar clinical signs, postmortem lesions and are both transmitted through aerosal route and contact between sick and healthy animals (Abd El-Rahim *et al*., 2010; Khan *et al*., 2012; Muse *et al*., 2012; Kgotlele *et al*., 2014b; Birindwa *et al*., 2017; Rahman *et al*., 2018), they have not been addressed together, so co-infections are not well established world-wide. There is a need to determine the risk factors that affect the spread of the two diseases in both goats and sheep for a better understanding of the the epidemiology of the diseases and plaaning for a better diseases control.

The surveillance and control strategies for diseases presenting with respiratory signs face challenges because the diagnosis in field depend on visual examination of the clinical signs and postmortem lesion whose accuracy are not validated and compounded by misdiagnosis (Halliday *et al*., 2012). In current situations, outbreaks of diseases with concurrent infections are reported to change clinical and postmortem presentatons, diseases dynamics and

outcomes depending on the concurrently infecting pathogens (Thumbi *et al.*, 2014; Gorsich *et al.*, 2017; Lello *et al.*, 2018). Concurrent infections in diseases presenting with respiratory signs results in altered presentation and persistence in the field, the persistence is reported to be longer with continued mortality (Kgotlele *et al*., 2018). The losses reported to have associated with diseases presenting with respiratory signs in small ruminants, the possible partial or misdiagnosis of specific disease(s) present in the outbreaks and accuracy of the diseases surveillance reports signifies the importance of further study in the area.

Establishing the distribution of the diseases, knowledge on concurrent infections and detailed documentation of clinical and postmortem lesions will improve the diagnosis and hence the accuracy of the reports used to inform policy and improve management. Therefore, the gathered information will help in improving policy, interventions and in putting appropriate control strategies.

1.4 Research objectives

1.4.1 General objective

The general objective of this study was to determine the epidemiology, field surveillance report gaps and concurrent infections for diseases manifesting with respiratory signs in small ruminants in different farming systems in Tanzania.

1.4.2 Specific objectives

(i) To determine the epidemiology and risk factors associated with diseases manifesting with respiratory signs in small ruminants in different livestock keeping systems in Tanzania.

(ii) To validate the accuracy of the existing field disease surveillance reporting system for diseases manifesting with respiratory signs in small ruminants in Tanzania.

(iii) To determine the concurrent infections in outbreaks of diseases which manifest with respiratory signs in small ruminants in Tanzania.

1.5 Research questions

(i) What is the epidemiology and risk factors associated with diseases manifesting with respiratory signs in small ruminants in different livestock keeping systems in Tanzania.

(ii) What is the accuracy and way of improvement of the existing field disease surveillance reporting system for diseases manifesting with respiratory signs in small ruminants in Tanzania.

(iii) What are the concurrent infections and the effect they result in dynamics and presentations in outbreaks of diseases which manifest with respiratory signs in small ruminants in Tanzania.

1.6 Significance of the study

This study expounds on the epidemiology, accuracy of diagnosis, reporting and surveillance, and level of pathogens involvement in the outbreaks of diseases presenting with respiratory signs in small ruminants. The results of the study will assist field officers in areas with limited laboratory diagnostic facilities to have a wider knowledge on the epidemiology, clinical signs and postmortem lesions for diseases and co-infections presenting with respiratory signs. Success in control of these diseases will result in enhanced livestock health and productivity and, hence economic stability and improved livelihood of many poor families in Tanzania.

1.7 Delineation of the study

This study focused on determing the risk factors for spread of CCPP and PPR in small ruminants in different farming systems in Tanzania. The study further validated the accuracy of the diseases surveillance reporting systems, performance of the field diagnosis forms and modified field diagnosis forms were validated against the molecular diagnosis results for diseases presenting with respiratory signs. Finally, the diseases concurrent infections, presentation and dynamics were determined for various concurrent infections. Therefore the results of this study are based on the questionnaire survey data and ELISA results from the laboratory, field and modified field forms results and molecular detection of *M. capripneumoniae*, *M. capri*, *M. capricolum*, *P. multocida*, *M. haemolytica* and SRMV using

molecular assays optimized and used to detection of these pathogens in in lung tissues, pleural fluid, lymphodes and nasal swabs from small ruminants presenting with respiratory signs from different reported outbreaks in Tanzania. The findings from this study were subjected to a major research design limitations which has been recommended in the chapter five of this document.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diseases of small ruminants

Small ruminants are important assets for food security and family livelihood resilience in many families in developing countries, especially in the advent of climate change due to their ability to graze in arid and semi-arid rangelands (FAO, 2013). However, the performance of small ruminants in developing countries is low when compared to developed countries and diseases are among the major factors considered responsible for the poor performance. Small ruminants succumb to a number of diseases; the major ones being CCPP and other pneumonias caused by mycoplasmas especially of the *M. mycoides* "cluster" (Pettersson *et al.*, 1996; Manso-Silván *et al.*, 2007), pneumonic pasteurellosis and pneumonic mannheimiosis caused by *P. multocida* and *M. haemolytica,* respectively (Mohamed & Abdelsalam, 2008; Abdullah & Chung, 2014). Peste des petits ruminants (PPR) caused by small ruminant morbillivirus (SRMV) is also prevalent in small ruminants keeping communities (Kgotlele *et al.*, 2018). Other diseases include Rift Valley fever, bluetongue, contagious ecthyma, Maedi-Visna and Nairobi sheep disease (Rahman *et al.*, 2011). Small ruminants are also affected by a number of internal parasites which include roundworms like *Haemonchus contortus, Ooesophagostomum columbianum*, *Trichostrongylus colubriformis* and *Ostertagia circumcincta*, tapeworms like *Moniezia* species and lungworms including *Dictyocaulus filaria, Protostrongylus rufescens* and *Muellerius capillaris* and protozoan parasites like coccidiosis (Ritchie *et al.*, 2012; Ibukun & Oludunsin, 2015; Zvinorova *et al.*, 2016). Mites (causing mange) and fleas are external parasites, infesting goats and sheep being (Kaufman *et al.*, 2012). Protozoan diseases of small ruminants include rickettsiosis and trypanosomiasis (Ibukun & Oludunsin, 2015; Velusamy *et al.*, 2015; El-Shahawy, 2016). Of all diseases of small ruminants, CCPP and PPR are among the major transboundary diseases (Seyoum & Teshome, 2017). Contagious caprine pleuropneumonia is highly contagious and fatal disease mainly of goats, localized, spreads aerosally and localizes in the respiratory system (Abd-Elrahman *et al.*, 2020) whereas, PPR spreads through nasal or oral route, invading nasopharyngeal and mesenteric lymph nodes and viruses are mainly in the digestive system, the end-stage bronchopneumonia is due to bacterial complication related to immunosuppression (Bamouh *et al.*, 2019), the major bacteria involved being *P. multocida* and *M. haemolytica* (Rawat *et al.*, 2019; Taunde *et al.*, 2019).

2.2 Contagious caprine pleuropneumonia and other mycoplasmal pneumonias

Contagious caprine pleuropneumonia is caused by *M. capripneumoniae,* which is a member of the "*Mycoplasma mycoides* cluster", a group of mycoplasmas that share many genotypic and antigenic characteristics (Manso-Silván *et al.*, 2007; Pettersson *et al.*, 1996). Members of the *M. mycoides* cluster are currently grouped into three subclusters comprising of the *Mycoplasma mycoides* subcluster, *Mycoplasma capricolum* subcluster and the nov. sp. *Mycoplasma leachii*. *Mycoplasma mycoides* subcluster has two species; *Mycoplasma mycoides* subsp. *mycoides,* the causative agent of contagious bovine pleuropneumonia (CBPP), and *Mycoplasma mycoides* subsp. *capri,* a newly designated species now incorporating the former *Mycoplasma mycoides* subsp. *mycoides* large colony type as a serovar. The *Mycoplasma capricolum* subcluster is comprised of *Mycoplasma capricolum* subsp. *capricolum* and *Mycoplasma capricolum* subsp. *capripneumoniae* the causative agent of CCPP. The last subcluster, nov. sp. *Mycoplasma leachii* is comprised of the group of previously unassigned *Mycoplasma sp*. Bov. Group 7 described by Leach (Manso-Silván *et al.*, 2009). *Mycoplasma capripneumoniae* can also infect sheep without development of a clinical disease (Litamoi *et al.*, 1990; Bölske *et al.*, 1995), but the role of sheep in the epidemiology of the disease has not been established.

Diagnosis of CCPP is difficult and cumbersome due to the fastidious nature and slow growth of *M. capripneumoniae* in culture media (Thiaucourt *et al.*, 1996). This slow growth results into a lengthy procedure and at times *M. capripneumoniae* being overgrown by other mycoplasmas. Advanced serological tests such as Latex Agglutination Test (March *et al.*, 2000) which is direct and can be performed at the field level or competitive Enzyme Linked Immunosorbent Assay (Peyraud *et al.*, 2014; Ahmad *et al.*, 2020) or gene based amplification of DNA from clinical samples using Polymerase Chain Reaction (PCR), Random Fragment Length Polymorphism (RFLP) or hybridization (Kusiluka *et al.*, 2001; Yatoo *et al.*, 2019) are also used in diagnosis. Because isolation is cumbersome and advanced techniques are difficult to use under field conditions, tentative diagnosis can be achieved basing on clinical signs, including severe respiratory distress, sero-mucoid nasal discharge, coughing, dyspnoea, pyrexia, pleurodynia and general malaise (FAO, 2015; Yatoo *et al.*, 2019a; OIE, 2019), pathological and histological features, such as severe lobar fibrinous pneumonia, profuse accumulation of straw coloured fluid in the chest cavity, severe congestion of the lungs, unilateral lung inflammation and lung attachment to the chest wall (Wesonga *et al.*, 2004;

Swai *et al.*, 2013; Yatoo *et al.*, 2019a). In control strategies, treatment with novel antibiotics such as fluoroquinolones, macrolides, tylosin and tetracycline can be of value at early diagnosis, but may result into carrier animals (Sadique *et al.*, 2012; Yatoo *et al.*, 2019a; Gal*Vmed*, 2018). Despite the use of antibiotics, dependence on therapeutic strategy as a control method for CCPP is currently not a viable option due to pathogen persistence, economic issues and concerns of antibiotic resistance and drugs residues in food chains while prophylactic and vaccinations are becoming more acceptable approaches (Yatoo *et al.*, 2019b). Vaccination of healthy animals is considered to be the best control method of the disease that provides protection of up to 100% (Giadinis *et al.,* 2008). However, the application and effectiveness of the vaccination approach in pastoral communities has been difficult to evaluate due to lack of data resulting from dynamics of the population, lack of knowledge of the livestock keepers and limited outreach, which justifies the need for national vaccination campaigns in promoting access to vaccines and vaccinations against CCPP (Wambura *et al.*, 2014; Renault *et al.*, 2019).

2.3 Pneumonic pasteurellosis and pneumonic mannheimiosis

Pneumonic pasteurellosis (PP) and pneumonic mannheimiosis (PM) mainly affect more the kids and lambs compared to adults (Tahamtan *et al.*, 2014), and occur after *P. multocida* or *M. haemolytica* gain access to the lungs when hosts' defenses are compromised due to other stress or infections (Jesse *et al.*, 2019). *Pasteurella multocida* and *M. haemolytica* belong to the genus *Pasteurella* originating from the previous *Pasteurella hemolytica* (*P. hemolytica*), which comprised of two biotypes, biotype-A which ferment L-arabinose and biotype-T which ferment trehalose. Currently, *P. hemolytica* is reclassified to a distinct species *Pasteurella trehalosi* (*P. trehalosi*) whereas, those that were trehalose negative were found to represent a distinct genus *Mannheimia* which comprised five species namely; *M. haemolytica*, *M. ruminalis*, *M. granulomatis*, *M. varigens* and *M. glucosida* (Jaworski *et al.*, 1998). *Pasteurella trehalose* was further reclassified to the genus *Bibersteinia* and named *Bibersteinia trehalosi* (Blackall *et al.*, 2007). The three species, *P. multocida, M. haemolytica*, and *B. trehalose* are important members of the family *Pasteurellaceae* known to pose serious health hazards in the livestock industry.

Diagnosis of PP and PM can presumptively rely on the clinical signs (Ekong *et al.*, 2014; Jesse *et al.*, 2015) and, postmortem and histological changes (Radi *et al.*, 2002; Rawat *et al.*, 2019). In some occasions, both PP and PM may result in sudden death (Assefa & Kelkay,

2018). On the other hand, in diagnosing PP and PM, the causative agents can be isolated from field samples in enriched blood agar media (Marru *et al.*, 2013; Rawat *et al.*, 2019). Advanced serological techniques, including indirect haemaglutination test (Berhe *et al.*, 2017; Assefa & Kelkay, 2019), and molecular techniques (Saed *et al.*, 2015; Rawat *et al.*, 2019) can be used. The causative agents are sensitive to a range of antibiotics, but drug sensitivity testing is recommended to determine effectiveness of the antibiotics in use. Control of PP and PM highly dependent on managing factors that predispose the animals to the disease (Forbes *et al.*, 2011), diversity in the circulating serotypes of both *P. multocida* and *M. haemolytica* is a big challenge in selecting serotypes with potential for vaccine development (Berhe *et al.*, 2017).

2.4 Peste des petits ruminants

A Peste des petits ruminant (PPR) is a disease that affects the digestive system and cause respiratory signs following secondary bacterial infection (Hamdy *et al.*, 1976; Bamouh *et al.*, 2019). It is caused by small ruminant morbillivirus (SRMV) formerly known as peste des petits ruminans virus (PPRV) (International Committee on Taxonomy of Viruses [ICTV], 2018), which is a single-stranded, negative sense, unsegmented RNA virus belonging to the genus *Morbillivirus* (Baron *et al.*, 2015). Small ruminants morbillivirus has four genetically distinct lineages (I-IV), which are geographically distributed, with lineages I and II being common in Western and Central Africa, lineage III in East Africa and lineage IV in the Middle East and Southern Asia (Banyard *et al.*, 2010).

Diagnosis of PPR can tentatively be reached relying on the clinical signs and postmortem lesions (Islam *et al.*, 2014; Bamouh *et al.*, 2019). Clinically, animals affected with SRMV manifest with pyrexia, severe depression, sneezing, dyspnoea, serous or mucopurulent oculo-nasal discharges, matting of the eyelids and blockage of nostrils, crackling lung sounds, focal necrotic stomatitis, halitosis, anorexia, mucoid diarrhea and sometimes tenesmus (Islam *et al.*, 2014; Kihu *et al.*, 2015; Bamouh *et al.*, 2019). Tentative diagnosis can be done basing on clinical signs, and postmortem lesions like ulcerative oral mucosa, reddened Payer's patches, swollen mesenteric and nasopharyngeal lymph nodes, congested large intestine and presence of zebra stripping in the ileocaecal junction which are pathognomonic lesions for PPR (Roeder & Obi, 2010; Truong *et al.*, 2014; Islam *et al.*, 2014). Severe broncho-interstitial pneumonia with macrophages within the lung alveoli and infiltration of macrophages in the sinuses of the spleen can also suggest presence of PPR under histopathological examination

(Chowdhury *et al.*, 2014; OIE, 2019). Definitive diagnosis for PPR can be reached by isolation of the SRMV from the field samples in cell lines such as Vero cells or BST-34 cell line which is more efficient (Latif *et al.*, 2018). Recent studies have also shown that adult kidney primary cell line can be used in the propagation of SRMV (Begum *et al.*, 2017). Evidence of the disease prevalence in non-vaccinated flocks can also be reached by use of serological tests such as cELISA (Abubakar *et al.*, 2017; Mopaco *et al.*, 2019), or confirmed by immunocapture ELISA (Sharawi *et al.*, 2010; Latif *et al.*, 2018). Peste des petits ruminants can also be confirmed using molecular techniques and a range of these techniques are available (Kinimi *et al.*, 2020). These include, Reverse-Transcriptase PCR (Kgotlele *et al.*, 2014; Shahriari *et al.*, 2019) or reverse transcription loop-mediated isothermal amplification and reverse transcription recombinase polymerase amplification assays (Kinimi *et al.*, 2020). Peste des petits ruminants (PPR) Global Eradication strategy focuses at eradication of the disease in the period of 15 years from 2015 (FAO & OIE, 2015). However, in the effort to eradicate PPR there are still gaps in epidemiological information, especially on the role of small ruminants movement and the role of abundant wildlife in the southern Africa where, veterinary services and vaccinations are limited (Britton *et al.*, 2019). For the efforts to be fruitful, regional cooperation in emphasizing on early detection, control of livestock movement, imposition of quarantine and mass vaccination of susceptible small ruminants is inevitable (Dilli *et al.*, 2011; Britton *et al.*, 2019; Fine *et al.*, 2020).

2.5 Risk factors for occurrence of diseases presenting with respiratory signs

Occurrence of CCPP and PPR are associated with various risk factors. In the previous studies the risk factors for occurrence of these diseases have been reported for each disease. In the study by Kipronoh *et al.* (2016), previous exposures to CCPP, distant sources of veterinary drugs, movements of goats to dry season feeding areas and markets as a source of new introductions to the flock were reported as the risk factors for the associated with CCPP. Occurrence of CCPP was also significantly associated with districts in the study carried out in Western Amhara in Northwestern Ethiopia, the other risk factors Age and sex were not significantly associated with CCPP (Abrhaley *et al.*, 2019). Other mycoplasmas like Mycoplasma agalactiae occurrence was associated with size of the herd, replacement of the farm animals and participation of animals in fairs and exhibitions (Matos *et al.*, 2019). Peste des petits ruminants (PPR) occurrence has been associated with females than males and goats than sheep in wildlife-livestock interphase (Rahman *et al.*, 2016), other reported factors were

seasons, being high in monsoon season, locations and road length and hence associated with livestock movements in Bangladesh (Rahman *et al.*, 2021). However, studies show that there are possibilities of co-occurrence of both CCPP and PPR in outbreaks presenting with respiratory signs (Kgotlele *et al.*, 2018). Little has been done on identifying risk factors that result in the occurrence of both diseases.

2.6 Co-occurrence of diseases presenting with respiratory signs and the effect on disease presentation

Aetiological agents involved in concurrent infections may act independently or interact with each other through various mechanisms, synergize or inhibit one another resulting into altered transmission dynamics, clinical presentation and severity and confounding effects of one aetiological agent to another at the individual or flock level (Thumbi *et al.*, 2014; Gorsich *et al.*, 2017; Lello *et al.*, 2018). Studies on multiple infecting parasites like *Theileria parva* and *Haemonchus contortus* (Thumbi *et al.*, 2014), co-infecting pathogens of bovine tuberculosis and brucellosis and how they scale up to produce population infection patterns (Gorsich *et al.*, 2017) and interacting nematodes effects (Lello *et al.*, 2018) showed changing dynamics of the resulting diseases. Concurrent exposure of the two major transboundary diseases CCPP and PPR have been reported by Kgotlele *et al.* (2018) in Tanzania. The outbreaks involved, despite causing significant losses due to mortality and reduced productions; they persisted for quite some time resulting in continuing losses to the farmers (Kgotlele *et al.*, 2018). Continued losses despite the interventions made to relieve PPR suggest presence of other un-attended pathogens that equally cause respiratory problems in goats and sheep and result in changing disease dynamics which need to be closely studied. Changing in disease presentations and severity were also reported in mixed infection of PPR and Capripox in Kivu, Democratic Republic of Congo (Birindwa *et al.*, 2017).

2.7 Prevention and control of diseases presenting with respiratory signs in small ruminants

Disease surveillance is very important in early detection which provides information on the spread of diseases, timely planning of the control programmes and mobilization of the resources (FAO, 1999; Kumar *et al.*, 2014; Chakraborty *et al.*, 2014). The Food and Agricultural Organization hence suggested the animal healthy delivery system for developing countries in an effort to strengthen the diseases surveillance (Fig. 1). Considering the

livestock movements and the nature of the livestock markets in Tanzania, vaccination against diseases is the best preventive option. Peste des petits ruminants, the most devastating disease in more than 70 countries in Africa and Asia, has been put in the programme for eradication through vaccination, the goal to be achieved in 15 years from 2015 (FAO & OIE, 2015). Similarly, vaccination has been shown to be the most effective method for control of CCPP. However, the use of vaccine against CCPP is still limited in Tanzania (Wambura *et al.*, 2014). Vaccines for control of pneumonic pasteurellosis and pneumonic mannheimiosis are available, but the major limitation is that, species exist in various strains such that vaccinations do not cross-protect and hence, vaccines need inclusion of strains available in the locality (Ayelet *et al.*, 2004; Tahamtan *et al.*, 2014) and other concurrent infecting pathogens in order to provide protection even in concurrent infections in small ruminants (Birindwa *et al.*, 2017; Kgotlele *et al.*, 2018). Therefore, this calls for further research on disease epidemiology and vaccinology.

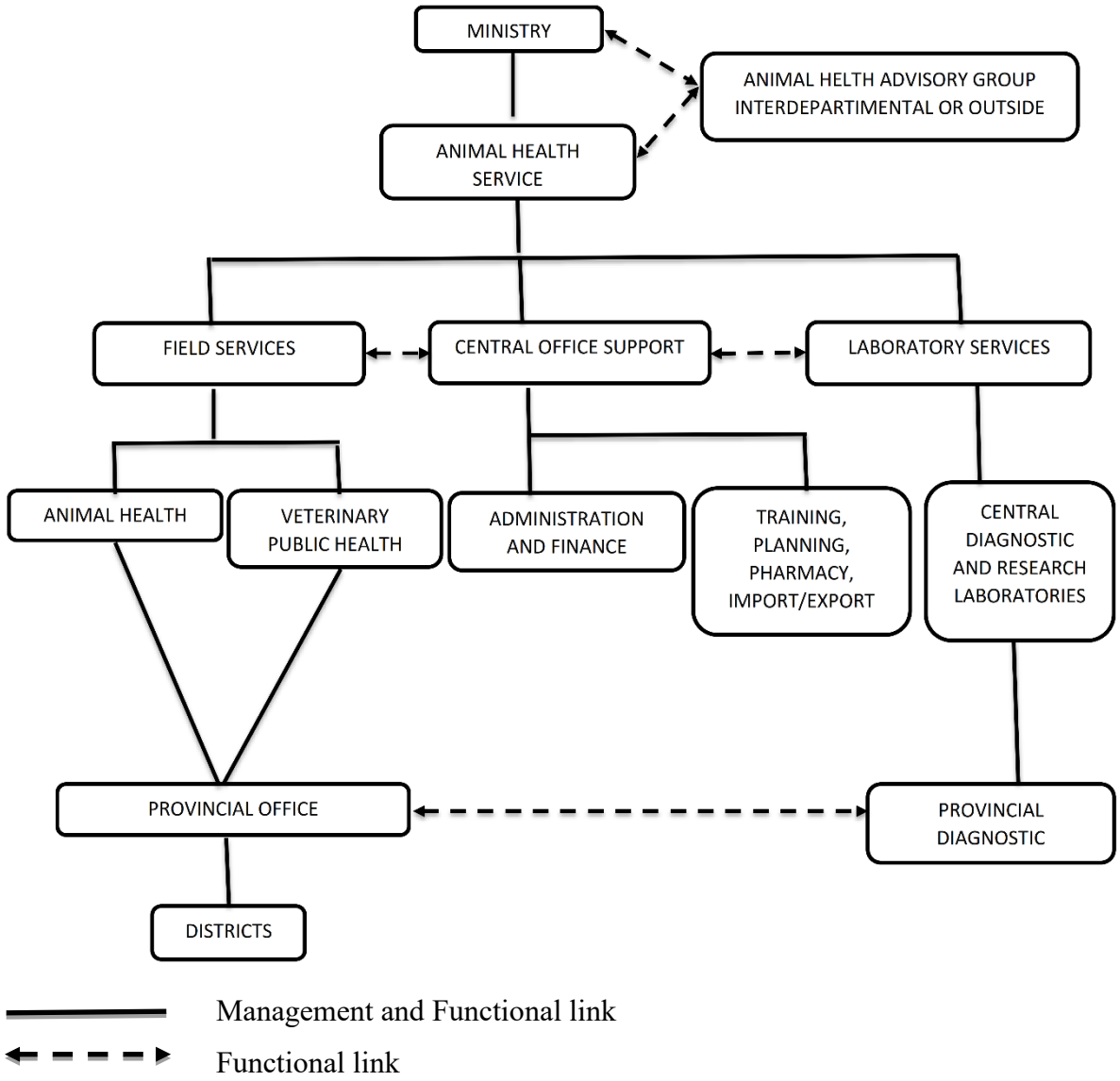

Figure 1: Model organogram for animal health services delivery (FAO, 1991)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Objective one

To determine the epidemiology and risk factors associated with diseases manifesting with respiratory signs in small ruminants in different livestock keeping systems in Tanzania.

3.1.1 Study areas

Administratively, Tanzania Mainland has 26 regions which are subdivided in seven agro-ecological zones, namely northern, lake, central, southern, southern highland, eastern and western zones (URT, 2014; Urassa, 2015). The zones and regions were randomly selected and the districts from each selected region were stratified into agropastoral and pastoral sampling frames. Districts and villages were stratified into those practicing agropastoral and pastoral farming systems and randomly selected from each sampling frame; households were selected following the guidance of the Livestock field officers (LFOs) and District Veterinary Officers (DVOs). The animals were stratified into goats and sheep and randomly selected from the bomas. In Tanzania flocks in agropastoral households are characterized by small flock sizes of animals ranging from 5 to 20, tethering of animals around the households, feeding the animals from the farm left overs and housing in bomas made of wooden or mud walls with corrugated iron sheet or thatched grass roofs whereas, pastoral flocks are characterized by large flock sizes of animals ranging from 30 to 300, fed in grazing lands, trekked for long distances in search for water and pasture, and kept in open kraals enclosed with wooden poles and thorny bushes. From each district three villages were randomly selected, some of the villages were close to wildlife protected areas (Fig. 2). Number of households practicing agriculture, percentages of households involved in livestock keeping and those practicing both crop farming and livestock keeping, and the livestock stocking density in the study areas are shown in Table 1.

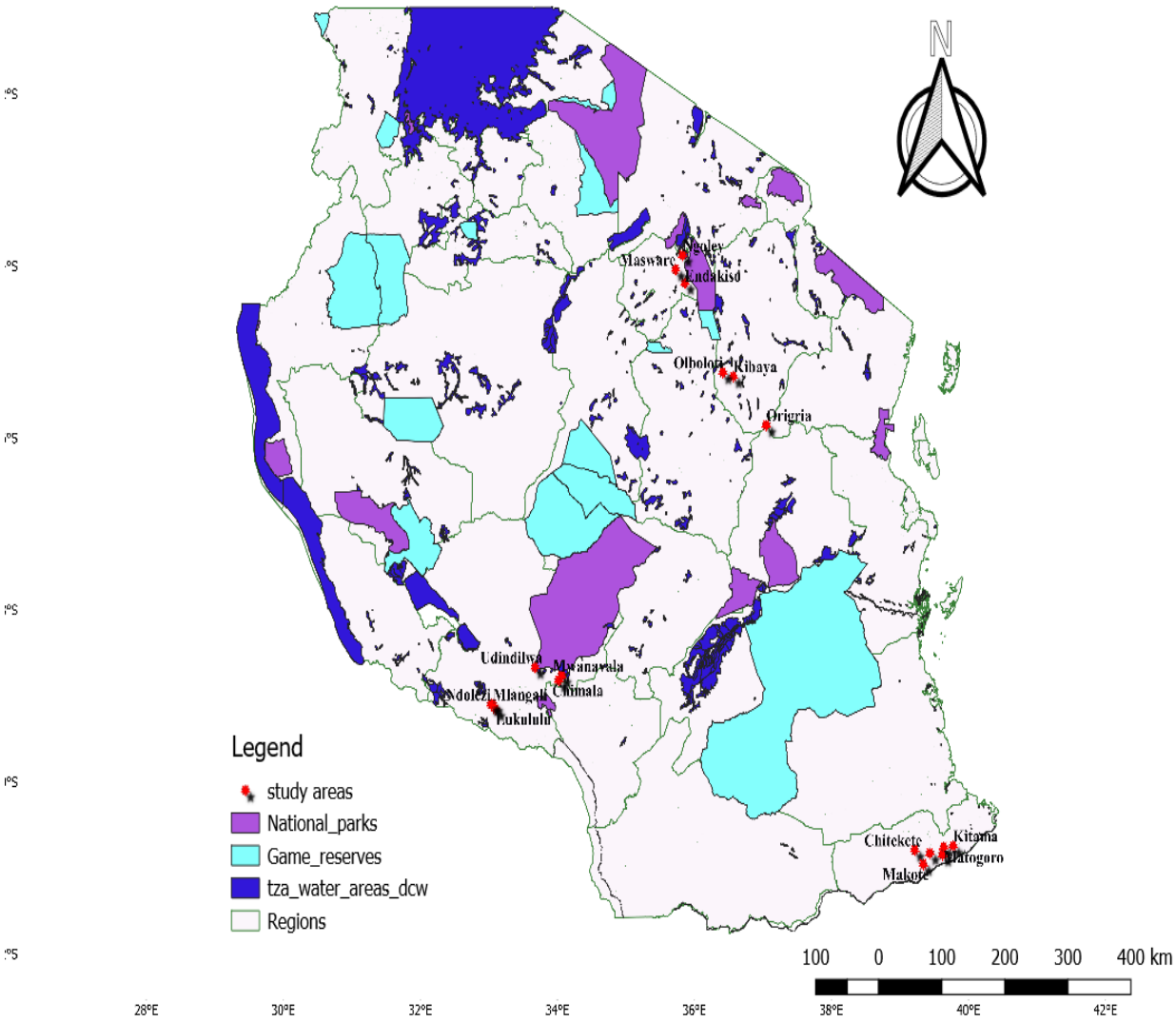

Figure 2: Map of Tanzania showing selected areas of the study

Table 1: Characteristic of the study regions in terms of human population and livestock density

Region	Total agricultural households	Households practicing mixed livestock and crop farming (%)	Households practicing only livestock farming only (%)	Average goats per household (flock)	Average sheep per household (flock)
Manyara	198 513	133 004 (67%)	5956 (3%)	13	9
Mbeya	372 844	139 444 (37.4%)	1119 (0.3%)	6	8
Mtwara	249 373	34 912 (14%)	324 (0.13%)	6	7

United Republic of Tanzania (2012, 2013)

3.1.2 Study design

A cross-sectional survey was conducted using randomized multistage stratified and random sampling strategy between June, 2016 and July, 2017 in three zones namely, northern, southern and southern highlands. Selected regions were Manyara, Mbeya and Mtwara from northern, southern highlands and southern zone, respectively. Depending on the type of farming system and the average flock size, two districts were randomly selected from each region. Selected districts and the systems were Babati district (agro-pastoral system) and Kiteto district (pastoral system) in Manyara region; Mbozi (agro-pastoral) and Mbarali (pastoral) in Mbeya region and; Newala (agro-pastoral) and Tandahimba (pastoral) in Mtwara region. Three villages were randomly selected from each district, making a total of 18 villages from both the agropastoral and pastoral farming systems. Seventy two households were selected for animal sampling, four (4) households from each village (Fig. 3). The lists of households were obtained from the district offices with the assistance of the DVOs or LFOs in the respective districts and villages.

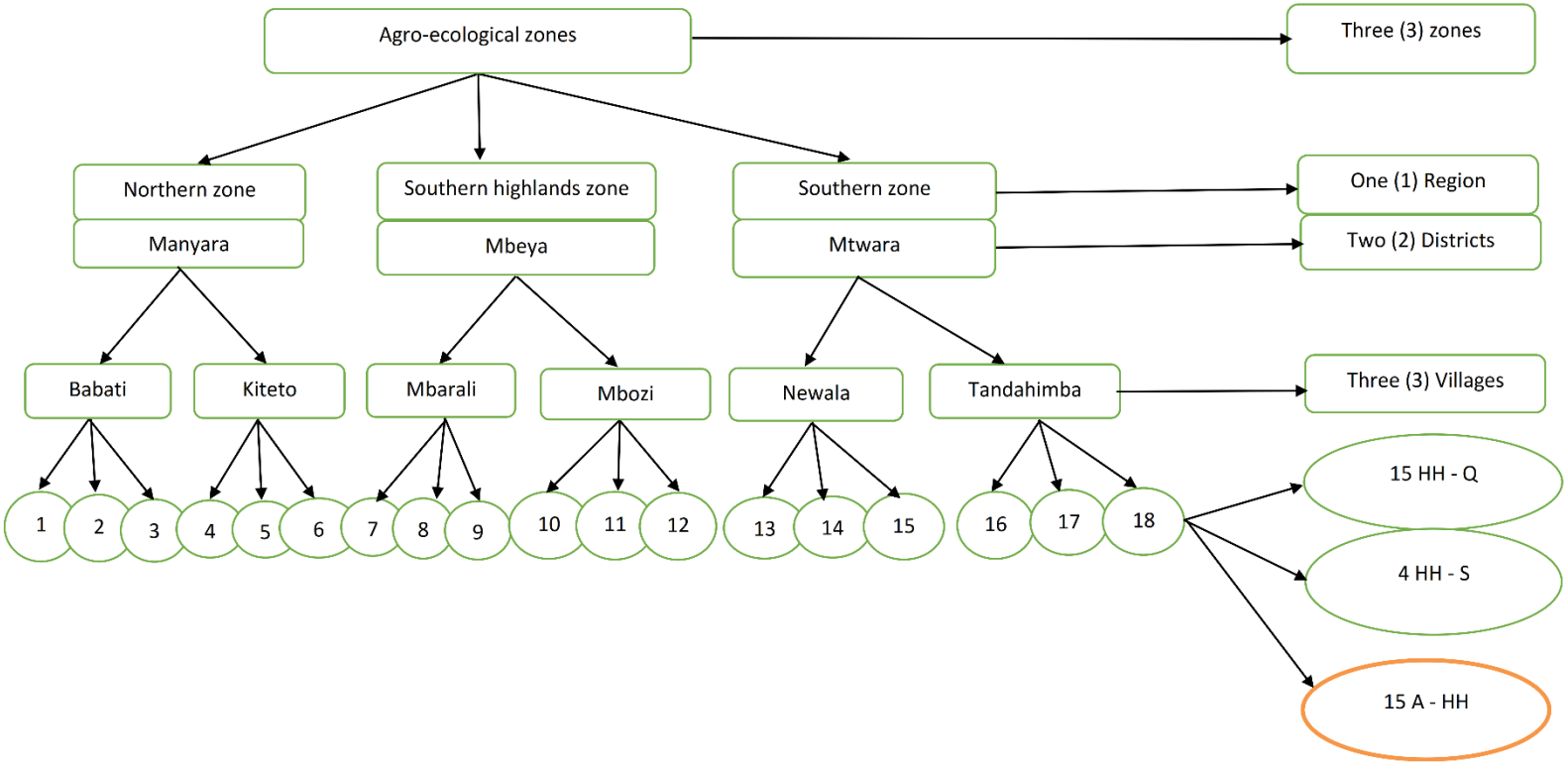

Figure 3: Study design

Legend

1……18 = Numbers representing the villages visited
15 HH – Q = Denotes the 15 households in which questionnaires were administered in each village
4 HH --- S = Denotes the 4 households from which animals were sampled in each village (comes from the 15HH-Q)
15 A – HH = Denotes the 15 animals that were sampled in each visited household in each village (comes from 4HH---S)

3.1.3 Sample size determination

Sample size was calculated using a formula for estimating sample size for cross-sectional studies as described by Charan and Biswas (2013). The formula used was $n=Z^2 \times pq/L^2$, where n is the sample size, Z is the standard normal variate, P an expected proportion based on previous studies, q equal 1-p and L an absolute error of precision. Previous prevalence of respiratory diseases/infections was estimated by the author to be 25% based on reports of previous prevalence of 22.10% for PPR (Kivaria *et al.*, 2013) and 26.4% for CCPP (Shija *et al.*, 2014), Z being 1.96, L equal 5% and q being 1-0.25. Then, the sample size calculated was 290 and to overcome for non-compliance and design effect, the sample size was increased by 10% making a sample size to be 320 and each agro-ecological zone was considered to be an independent population. A total of 960 samples were targeted for this study based on assumption of sampling from approximately 10 – 15 goats and sheep per household.

3.1.4 Data collection through questionnaires

A semi-structured questionnaire was prepared for obtaining data from the livestock keepers in this study. The questionnaire was designed to obtain information on risk factors for occurrence of respiratory diseases according to livestock keepers, livestock keepers' knowledge, attitude and perceptions on possible risk factors for transmission of diseases presenting with respiratory signs, emphasis being on CCPP and PPR. The risk factors included; farming system, outbreak of diseases presenting with respiratory signs in past 12 months, mixing of species (goats and sheep), mixing of flocks (mixing small ruminants from different flocks), mixing sick and healthy animals, selling and buying small ruminants in animal markets, grazing in the same land with wild animals and access to veterinary services. Questionnaires were also designed to record household demographic characteristics including the gender of the head of household, age of the head of household and the level of education from university to informal education which are obtained out of normal classes. The questionnaire was pre-tested in randomly selected livestock keeping households in Arusha and Pwani regions which were outside the study areas. The pre-test aimed at establishing the duration taken for questionnaire administration, questions format, respondents' reaction to questions, and whether the questions were understandable by the respondents.

3.1.5 Collection of blood samples

Blood samples were collected in plain vacutainer tubes (Neomedic Limited, Hertfordshire United Kingdom) through jugular vein puncture, allowed to clot and serum was decanted into 2 ml cryovials. The samples were kept in cool boxes with ice packs and transported to the zonal laboratories where they were frozen at -20 ^{0}C before being transported in iced cool boxes to the NM-MAIST laboratory where they were stored until used for serological analysis.

3.1.6 Serological testing

A commercial competitive Enzyme Linked Immunosorbent Assay (cELISA), ID Screen® (Lot 652-009 from ID.vet Innovative Diagnostics, Montpellier, France) was used to determine presence of SRMV antibodies in collected serum samples. Tests were carried out according to manufacturers' instructions. Both positive and negative controls were supplied by the manufacturer as part of the test kit. The mean positive and negative controls were each used to optimize the test. The optical density of each serum sample from each animal was used together with mean of the negative control to calculate the competitive percentage. A sample was considered positive if the competition percentage (ODsample/OD_{NC} x 100) was ≤ 50% (Libeau *et al.*, 1995).

Mycoplasma ELISA kit (Confidence™ IDEXX, Lot 306-5 from IDEXX Laboratories, Montpellier SAS, France) was used to test presence of antibodies against *M. capripneumoniae*. Tests were carried out according to manufacturer's instructions. Positive and negative controls, conjugates and monoclonal antibodies (Mab) 4.52 were supplied by the manufacturer as part of the kits. The means of the conjugate control mean of the monoclonal antibody (Mab) 4.52, and means of the positive and negative controls were calculated to establish the validity of the test. The sample percentage inhibition was calculated from the sample OD, mean monoclonal antibody (Mab) 4.52 and mean conjugate control absorbance. Sample's percentage of inhibition (S PI) was calculated using the formula: S PI% = 100 x (MabCẍ-SA (450)) / (MabCẍ-CCẍ), where MabCẍ is the Mab Control Mean Absorbance, SA is the Sample Absorbance, and CCẍ is the Conjugate Control Mean Absorbance. A sample was considered positive when S PI ≥ 55% (Thiaucourt *et al.*, 1994; Peyraud *et al*., 2014).

3.1.7 Data management and statistical analysis

Questionnaire data were entered in Microsoft Excel® 2007, cleaned and checked for errors. The analysis were later carried out and analysed to provide the characteristics and outcomes across the study districts. *Mycoplasma capripneumoniae* and SRMV seropositivity status at flock level was established using descriptive statistics with 95% Confidence Interval (95%CI). Univariable analysis for dichotomous variables was carried out in R, version R 3.5.1 (R Core Team, 2018) using generalized linear models (glm). The significance level was set at $p< 0.25$ (Dohoo *et al.*, 2009) for univariate analysis.

$$model0 = glm(outcome\ variable(disease) \sim explanatory\ variable(risk\ factors))$$

A multivariable logistic regression model was then built using significant risk factors from the univariable analysis described by Hosmer and Lemeshaw (1989), to develop a final model of risk factors influencing *M. capripneumoniae* and SRMV seropositivity at $p \leq 0.05$.

$$model0 = glm(outcome\ variable(disease) \sim explanatory\ variable\ (risk\ factor1) + \cdots Explanatory\ variable(risk\ factor(n)))$$

The strength and direction of the risk factors for *M. capripneumoniae* and SRMV seropositivity in small ruminants were estimated using odds ratios (OR), 95% Confidence interval and *p values* for the final model were computed using the formula.

$$\exp(cbind(OR = coef(model0), confint(model0))$$

3.2 Objective two

To validate the accuracy of field disease surveillance reporting system on diseases manifesting with respiratory signs in small ruminants in Tanzania.

3.2.1 Study areas

The areas involved in the study were all those from which classic outbreaks and sporadic outbreaks of diseases presenting with respiratory signs were reported in the course of this study. Outbreak reports from districts in the Southern Highland Zone including Chunya and Mbarali districts in Mbeya region and Iringa Urban and Kilolo districts in Iringa region were received and follow up investigation was made. In the Central Zone, Dodoma Urban district was involved, whereas in the Eastern Zone, reports from Mvomero district in Morogoro

region and Bagamoyo district in the Coast region were received and followed up. In the Lake Zone, outbreak reports from Serengeti district were studied while in the Northern Zone; reports from Korogwe in Tanga region and Monduli in Arusha region were included in the study (Fig. 4).

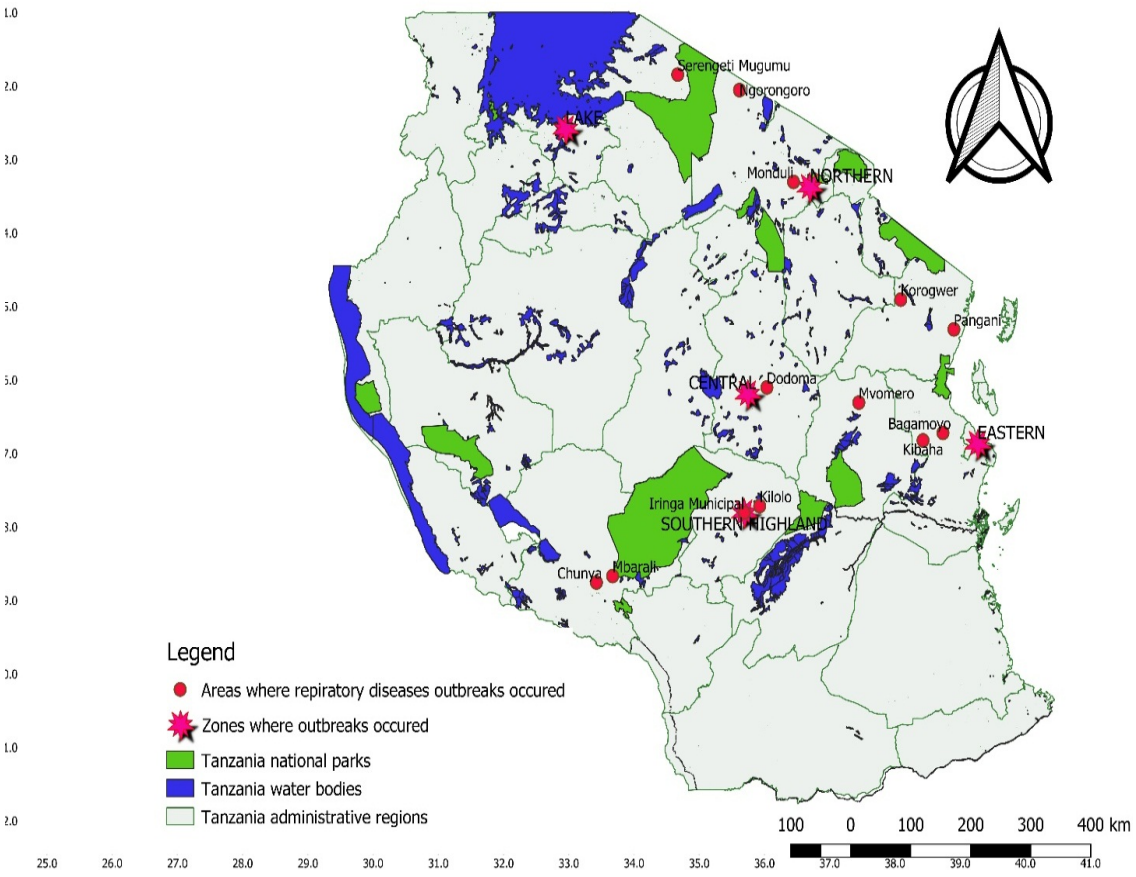

Figure 4: Map of Tanzania showing zones and districts that reported outbreaks of diseases with respiratory signs in small ruminants between June, 2016 and July, 2018

3.2.2 Study animals

The study animals included clinically sick goats and sheep from the flocks which had outbreaks of diseases presenting with respiratory signs in different zones of Tanzania.

3.2.3 Study approach

An outbreak investigation/surveillance approach was used where a total of 205 cases were investigated between June, 2016 and July, 2018. In each outbreak, an investigation was carried out following the steps in outbreak investigation approach as described by Huang and

Bayona (2004). Briefly, the preparations for the investigation were made after, the outbreaks existences were verified and case definitions were made. Descriptive epidemiology to determine the personal characteristics of the cases, changes in disease frequency over time, and differences in disease frequency based on location were established. Hypotheses about the cause or source were developed and evaluated, investigations were conducted and finally, results were communicated.

3.2.4 Field surveillance

Routine field surveillance forms that are used in disease surveillance in Tanzania contain several information including; provisions for recording the production systems, major clinical and postmortem features (Appendix 2). The routine surveillance forms were used by the LFOs or DVOs during outbreak investigations to establish tentative diagnosis and for the purpose of this study, these forms were referred to as Field Data Form One (FD-1). With respect to diseases manifesting with respiratory signs, FD-1 was modified by the researcher by listing the probable clinical signs and postmortem lesions based on the knowledge of diseases presenting with respiratory signs (Appendix 3). The modified forms, hereinafter referred to as Field Data Form Two (FD-2) were used by researchers to make tentative diagnosis during outbreak investigations. From each of the animals examined, the samples collected from clinically sick animals were whole blood, nasal, ocular, and oral swabs, whereas, from autopsied animals, lung tissues sections, synovial fluid, lymph nodes sections, intestinal sections and pleural fluids were collected for laboratory diagnosis. Sampling and field investigations were conducted by the DVOs and LFOs using the guidance of FD-1 and FD-2 for each case.

3.2.5 Field data and samples collection

Samples were collected during clinical examination in EDTA vacutainer tubes RNA shields (Zymo Research Corporation, Irvin, California) and kept in iced cool boxes from the field to the district laboratory for storage at -20 ^{0}C. From autopsied animals samples were collected in Stuart Medium and kept in iced cool boxes from the field to the district laboratory for storage at -20 ^{0}C.

All samples were later transported in cool boxes to the Nelson Mandela African Institution of Science and Technology laboratory. Samples in RNA shield and Stuart Medium were kept at -20 ^{0}C while blood samples were kept at 4 ^{0}C prior to analysis.

3.2.6 Laboratory analysis

(i) DNA and RNA extraction

Genomic DNA (gDNA) was extracted using *Quick*-DNA™ Miniprep plus Kit Catalog No D4069 (Zymo Research Corporation, Orange, California) from the lung tissue sections, pleural fluids and nasal swabs as per manufacturers' instructions on solid and biological fluids and cell extractions. In extracting gDNA, pleural fluids were mixed with BioFluids and cell buffer supplied in the kit whereas, solid tissues were minced and mixed with solid tissue buffer, then digested with Proteinase K at 55 ^{0}C. Then each preparation, from pleural fluid or lung sections were mixed with genomic binding buffer and transferred to the spin column for washing and elution.

The RNA was extracted using *Quick*-RNA™ Viral Kit Catalog No R1035 (Zymo Research Corporation, Orange, California). For RNA extraction, equal amounts of the sample in RNA shield, were added to Beta mercaptoethanol to a final dilution of 0.5% (v/v), transferred to the spin column and washed by viral wash buffer. Then the viral wash buffer was removed using pure ethanol and eluted in DNase/RNase free water.

Extracted RNA and DNA were briefly kept at -80 ^{0}C before amplification of the SRMV nucleoprotein (N) gene from RNA and, *M. mycoides* "cluster" 16S rRNA gene and specific gene for *M. capri*, *M. capricolum*, *P. multocida* and *M. haemolytica* from gDNA. Set of primers used are listed in Table 2.

Table 2: Set of primers used in the identification of target pathogen involved in outbreaks of diseases presenting with respiratory signs

Pathogen	Primer set	Amplified gene	Band size	Reference
M. mycoides "cluster"	CAF5'-CGA AAG CGG CTT ACT GGC TTG TT-3'	16S rRNA	540 bp	Bascunana *et al.* (1994)
	CAR5'-TTG AGA TTA GCT CCC CTT CAC AG-3'			
M. *capricolum*	F5'-AGA CCC AAA TAA GCC ATC CA-3'	*LppA*	1356 bp	Monnerat *et al.* (1999)
	R5'-CTT TCA CCG CTT GTT GAA TG-3'			
M. capri	P4 5'-ACT GAG CAA TTC CTC TT-3'	CAP-21 gene	395 bp	Hotzel *et al.* (1996)
	P6 5'-TTA AAT AAG TTT GTA TAT GAA T-3'			
SRMV	NP3-5- TCT CGG AAA TCG CCT CAC AGA CTG -3	Nucleoprotein (N) gene	351 bp	Kgotlele *et al.* (2014b)
	NP4 -5- CCT CCT CCT GGT CCT CCA GAA TCT -3			
M. haemolytica	MHSSA-5'-TTC ACA TCT TCA TCC TC-3'	SSA- 1 gene	325 bp	Hawari *et al.* (2008)
	MHSSA-5'TTT TCA TCC TCT TCG TC-3'			
P. multocida	PmOUT-5'-AGG TGA AAG AGG TTA TG-3'	Outer membrane	219 bp	Hawari *et al.* (2008)
	PmOUT-5'-TAC CTA ACT CAA CCA AC-3'	protein 87 (Omp87).		

(ii) Detection of Mycoplasma capricolum subsp capripneumoniae

Molecular detection of the *M. capripneumoniae* was carried out using PCR/REA technique (Bölske *et al.*, 1996). The 16S rRNA gene for members of the *M. mycoides* "cluster" was amplified by PCR. The amplification process was carried out using the set of primers as described by Bascunana *et al.* (1994) and listed in (Table 2). Master Mix, PCR water and the template were mixed to 25 µl total volume, preheated in C100 touch thermal cycler (BIO-RAD®, Singapore) at 94 ^{0}C for 5 minutes. After initial preheating, 40 PCR normal cycles followed and conditions were denaturation at 95 ^{0}C for 1 minute, annealing at 58 ^{0}C for 1 minute and elongation at 72 ^{0}C for 2 minutes. The thermal cycler machine was set to holding temperature of 72 ^{0}C for 10 minutes and 4 ^{0}C to infinite after completion of the 40 normal cycles. The PCR products were visualized in 0.5% DNA loading dye (EZ-Vision®, VWR Life Science, California, USA) in 1.5% agarose, after gel electrophoresis. The amplicons were digested by restriction enzyme *PstI*, resulting into three fragments of 548 bp of the operon that was not ligated, 420bp and 128bp from the ligated operon indicating presence of *M. capripneumoniae.* The other members of the *M. mycoides* "cluster" had only two fragments 420bp and 128bp from ligated operons of the 16S rRNA gene fragment (Bölske *et al.*, 1996).

(iii) Detection of other members of the Mycoplasma mycoides "cluster"

Mycoplasma capricolum subsp *capricolum*, another member of the *Mycoplasma mycoides* "cluster" in the same subcluster with *M. capripneumoniae* was detected by amplification of *LppA* gene using specific primers listed in (Table 2) as described by Monnerat *et al.* (1999). The amplification cycle was done in 25 µl, in a thermocycler which was preheated at 94 ^{0}C for 2 min, then, followed by 35 normal cycles of denaturation at 94 ^{0}C for 30 seconds, annealing at 51 ^{0}C for 30 seconds, and extension at 72 ^{0}C for 60 seconds. The amplified gene fragment expected size was 1356 bp (Monnerat *et al.*, 1999). The other member of the *M. mycoides* "cluster" from the *M. mycoides* subcluster, *M. capri* (Manso-Silván *et al.*, 2009) was detected by amplification of the CAP-21 gene using specific primers listed in (Table 2) as described by Hotzel *et al.* (1996).

(iv) Detection of *Pasteurella multocida* and *Mannheimia hemolytica*

Detections of *M. haemolytica* and *P. multocida* were done by amplification of the serotype specific antigen 1 (SSA-1) and outer membrane protein 87 (Omp87) genes, respectively

(Hawari *et al.,* 2008). The amplification process was done in C100 touch thermal cycler (BIO-RAD®, Singapore), in 25 µl total volume of substrate, Master Mix, PCR water using specific primers listed in (Table 2). The thermocycler was preheated at 94 ^{0}C for 2 minutes, then, followed by 40 normal cycles of denaturation at 94 ^{0}C for 45 seconds, annealing at 45 ^{0}C for 45 seconds, and elongation at 72 ^{0}C for 1 minute. The resulting amplicons were detected in 1.5% agarose gel stained with 0.5% DNA loading dye (EZ-Vision®, VWR Life Science, California, USA).

(v) Detection of Small Ruminants' Morbillivirus (SRMV)

Small ruminants' morbillivirus, a member of the genus *Morbillivirus* and causative agent of peste des petits ruminants was detected by amplification of the Nucleoprotein (N) gene using specific NP3/NP4 (Table 2) in one-step Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) as described by Couacy-Hymann *et al.* (2002). A reverse transcription was carried at 45 ^{0}C for 30 min, then initial denaturation at 95 ^{0}C for 30 seconds which was followed by 40 cycles of denaturation at 95 ^{0}C for 30 seconds, annealing at 55 ^{0}C, elongation at 72 ^{0}C, held at 72 ^{0}C for 10 minutes. The resulting amplicon was then run in the 1.5% agarose gel electrophoresis, stained with 0.5% DNA loading dye (EZ-Vision®, VWR Life Science, California, USA). The resulting amplicon band size was 351bp (Couacy-Hymann *et al.*, 2002; Kgotlele *et al.*, 2014b).

3.2.7 Data management and statistical analysis

Estimates of proportions of positive cases over the sampled cases were calculated using R version R. 3.5.1 (R Core Team, 2018). In order to obtain factual information in point and proportions estimation, stratification by species and surveillance zones was incorporated.

Sensitivity and specificity of the results obtained using FD-1 and FD-2 were validated by using the results obtained from the gold-standard molecular laboratory results. Sensitivity indicated that the probability that the test will capture the "diseased" among those subjects with a disease whereas specificity indicated fractions of those without disease who will have negative test results. These were calculated using the formula as described by Hernaez and Thrift (2017).

$$Sensitivity = A/\ (A + C) * 100$$

Where *"A"* equals to *True positive* and *"C"* equals to *False negative*

$$Specificity = D/\ (D + B) * 100$$

Where *"D"* equals to *True negative* and *"B"* equals to *False positive*

On the other hand, the positive and negative predictive values for the compared tests were also calculated.

$$Positive\ predictive\ value = A/(A+B) * 100$$

Where *"A"* equals to *True positive* and *"B"* equals to *False positive*

$$Negative\ predictive\ value = D/(D+C) * 100$$

Where *"D"* equals to *True negative* and *"C"* equals to *False negative*

Inter-rater reliability or test agreement was used to compare the performance of the FD-1 and FD-2 against LD was established by calculating the *Cohen's Kappa Coefficient* (κ). Obtained *Cohen's Kappa Coefficients* were interpreted as indicated by Landis and Koch (1977), that,

<0.00 is *poor,* 0.00 – 0.2 is *slight*, 0.21 – 0.4 is *fair*, 0.41 -0.60 is *moderate,* 0.61 – 0.80 is *substantial* and 0.81 – 1.00 is *almost perfect*.

$$Kappa\ coefficient = Pr(a) - Pr(c)\ /1 - Pr(c)$$

Where *"Pr (a)"is the proportions for agreement between FD-1 or FD-2 and LD and Pr (c) is the proportions for the agreement by chance between FD-1 or FD-2 and LD.*

3.3 Objective three

To determine the existence of concurrent infections in disease syndromes manifesting with respiratory signs in small ruminants in Tanzania.

3.3.1 Data source and extraction

Data used in establish the levels of concurrent infections were obtained from the outbreak investigation as described in objective 2. The data were from different agroecological zones inveolved in the outbreak investigations. Each case was examined for presence of multiple pathogens which cause the outbreaks of diseases presenting with respiratory signs in goats and sheep.

3.3.2 Data Management and statistical analysis

The results from the Microsoft Excel®2010 obtained from the outbreak investigation as described in objective 2 were used to establish the cases with concurrent infections. The columns with laboratory results for CCPP, *M. capri* pneumonia, *M. capricolum* pneumonia,

P. multocida pneumonia, *M. haemolytica* pneumonia and SRMV were used to identify cases that had multiple infections. Proportions were calculated in R version R. 3.5.1 (R Core Team, 2018). The obtained data were from goats and sheep showing clinical signs and postmortem lesions suggestive of respiratory system involvement.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Demographic data and seroprevalence of *M. capripneumoniae* and SRMV in goats and sheep

4.1.1 Demographic characteristics and flock composition in the study districts

A total of seventy (70) households keeping goats (31.4%), sheep (5.7%) or both (62.9%) were involved in the study with the majority (84.3%) coming from agropastoral farming system. Gender distribution showed that 88.6% (62/70) of the households were led by males with 82.9% (58/70) having primary level education. Exceptions were observed in Mbarali district where there were farmers with secondary 7.1% (1/12) and tertiary education 7.1% (1/12). There were also no head of households with informal education in Mbozi, Newala and Tandahimba as shown in Table 3.

Table 3: Household characteristics in the study districts

Variable	Category	Frequency (%) (*n = 70*)	Manyara		Mbeya		Mtwara	
			Babati *n=12*	**Kiteto** *n=11*	**Mbarali** *n=14*	**Mbozi** *n=11*	**Newala** *n=12*	**Tandahimba** *n=10*
Head of household gender	Females	8 (11.4%)	2 (16.7%)	1 (9.1%)	0 (0.0)	1 (9.1%)	2 (16.7%)	2 (20%)
	Male	62 (88.6%)	10 (83.3%)	10 (90.1%)	14 (100%)	10 (90.1%)	10 (83.3%)	8 (80%)
Farming system	Pastoral	11 (15.7%)	0 (0.0)	1 (9.1%)	7 (50%)	2 (18.2%)	1 (8.3%)	0 (0.0)
	Agropastoral	59 (84.3%)	12 (100%)	10 (90.9%)	7 (50%)	9 (81.8%)	11 (91.7%)	10 (100%)
Level of education	Informal	8 (11.4%)	4 (33.3%)	1 (9.1%)	3 (21.4%)	0 (0.0)	0 (0.0)	0 (0.0)
	Primary	58 (82.9%)	8 (66.7%)	10 (90.9%)	9 (64.3%)	10 (90.9%)	12 (100%)	9 (90%)
	Secondary	3 (4.3%)	0 (0.0)	0 (0.0)	1 (7.1%)	1 (9.1%)	0 (0.0)	1 (10.0%)
	Tertiary	1 (1.4%)	0 (0.0)	0 (0.0)	1 (7.1%)	0 (0.0)	0 (0.0)	0 (0.0)
Flock composition	Both	44 (62.9%)	11 (91.7%)	8 (72.7%)	12 (85.7%)	6 (54.5%)	2 (16.7%)	5 (50%)
	Goats	22 (31.4%)	1 (8.3%)	2 (18.2%)	2 (14.3%)	4 (36.4%)	9 (75%)	4 (40%)
	Sheep	4 (5.7%)	0.(00)	1 (9.1%)	0 (0.0)	1 (9.1%)	1 (8.3%)	1 (10%)

4.1.2 Seroprevalence across the study districts

Overall seroprevalences of *M. capripneumoniae* and SRMV antibodies in goats were 6.5% (44/676) and 28.7% (193/676), respectively; whereas, in sheep the overall seroprevalences were, 4.2% (12/285) for *M. capripneumoniae* antibodies and 31.9% (91/285) for SRMV antibodies. The overall seroprevalences of both *M. capripneumoniae* and SRMV antibodies were 2.7% (18/676) and 2.1% (6/285) in goats and sheep, respectively. In all the study districts, SRMV seropositivity was higher compared to *M. capripneumoniae* seropositivity. On the other hand, SRMV seropositivity was higher in goats compared to sheep in Kiteto, Newala and Tandahimba districts. Seropositivity to *M. capripneumoniae* was higher in goats as compared to sheep in all study districts except Tandahimba as given in Table 4.

Table 4: Distribution of *M. capripneumoniae* and SRMV antibodies seropositivity by species and districts

Species studied	Description	Overall	Manyara		Mbeya		Mtwara	
			Babati	Kiteto	Mbarali	Mbozi	Newala	Tandahimba
Goats	Sampled	676	107	119	136	100	127	87
(n=676)	Seropositive to *M. capripneumoniae*	44 (6.5%)	16 (15%)	7 (5.9%)	5 (3.7%)	6 (6.0%)	3 (2.4%)	7 (8.1%)
	Seropositive to SRMV	193 (28.6%)	25 (23.4%)	39 (32.8%)	52 (37.4%)	17 (17%)	31 (24.4%)	29 (33.3%)
	Seropositive to both	18 (2.7%)	7 (6.5%)	3 (2.5%)	1 (0.7%)	2 (2.0%)	2 (1.6%)	3 (3.5%)
Sheep	Sampled	285	55	64	77	23	33	33
(*n = 285*)	Seropositive to *M. capripneumoniae*	12 (4.2%)	4 (7.3%)	3 (4.7%)	*Nil*	*Nil*	*Nil*	5 (15.2%)
	Seropositive to SRMV	91 (31.9%)	17 (31%)	17 (26.6%)	34 (44.2%)	12 (52.2%)	3 (9.1%)	8 (24.2%)
	Seropositive to both	6 (2.1%)	2 (3.6%)	1 (1.6%)	*Nil*	*Nil*	*Nil*	3 (9.1%)

4.2 Risk factors for diseases manifesting with respiratory signs in small ruminants in different livestock management systems in Tanzania

4.2.1 Risk factors associated with *M. capripneumoniae* and SRMV antibodies seropositivity in small ruminants

(i) Univariate logistic regression analysis of risk factors associated with *M. capripneumoniae* and SRMV antibodies seropositivity in goats

The results of the univariate logistic regression showed that, farming systems significantly influenced differently the perpetuation of *M. capripneumoniae* and SRMV in goats. Mixing of flocks, mixing of species and grazing with wild animals were found to be significantly associated with higher seroprevalence of *M. capripneumoniae* and SRMV antibodies in the flocks. Provision of extra feeds at home and introduction of new animals in the past 12 months were significantly associated with higher SRMV seropositivity as shown in Table 5 and Table 6.

Table 5: Univariate logistic regression analysis of risk factors associated with *M. capripneumoniae* antibodies seropositivity in goats

Description	Risk factor	Odds Ratio	95% CI	*p value*
M. capripneumoniae antibodies seropositivity	Farming systems-pastoral	0.9472466	0.8997396- 0.997262	0.0394
	Health status - sick	1.204437	0.9451573– 1.534842	0.133
	Provision of extra feeds at home	1.037515	0.9874217– 1.090150	0.145
	Presence of sick animals in the past six months	1.030325	0.9818228– 1.081223	0.2251
	Mixing of species	1.07107076	1.0196102– 1.124492	0.00635
	Mixing of flocks	1.044938	1.005894– 1.085498	0.0240
	Grazing in areas where wild anmals graze	1.039888	1.001747– 1.079482	0.0406
	Introducing new animals in the past twelve months	0.9736485	0.9316836 - 1.017504	0.235
	Access to veterinary services	0.9564506	0.921611 - 0.992606	0.019
	Presence of ppr	1.040222	0.9983095 - 1.083894	0.0606

Table 6: Univariate logistic regression analysis of risk factors associated with SRMV antibodies seropositivity in goats

Description	Risk factor	Odds Ratio	95% CI	*p-value*
SRMV antibodies seropositivity	Age - young	0.916717	0.85554 - 0.982264	0.0138
	Farming system - pastoral	1.176551	1.071311 - 1.292130	0.000712
	Provision of extra feeds at home	1.184597	1.082810 - 1.295952	0.000237
	Presence of sick animals in the past six months	1.062678	0.9729047 - 1.160734	0.177
	Presence of outbreak in the past twelve months	1.055853	0.9837912 - 1.133194	0.132
	Mixing of species	1.057443	0.9663994 - 1.157064	0.224
	Mixing of flocks	1.096637	1.022866 - 1.175729	0.00963
	Grazing in areas where wild anmals graze	1.118259	1.044631 - 1.197077	0.00136
	Mixing health and sick animals of the same flock	1.051614	0.9823794 - 1.125728	0.148
	Introducing new animals in the past twelve months	0.8990737	0.82966 - 0.97429	0.00966
	Access to veterinary services	0.9606313	0.897348 - 1.028377	0.248
	Presence of CCPP	1.141328	0.9943443 - 1.310038	0.0606

(ii) Multivariate logistic regression analysis of risk factors associated with *M. capripneumoniae* and SRMV antibodies seropositivity in goats

Multivariate logistic regression analysis showed that *M. capripneumoniae* seropositivity was significantly low in pastoral farming and high when goats were mixed with sheep and grazed in the areas where wild animals graze. The risk factors that were significantly associated with SRMV perpetuated the spread of the disease as shown in Table 7.

Table 7: Multivariate logistic regression analysis of risk factors associated with *M. capripneumoniae* and SRMV antibodies seropositivity in goats

Description	Variable	Odds Ratio	95% CI	*p-value*
M. capripneumoniae antibodies seropositivity	Farming system -pastoral	0.91073332	0.8624517– 0.961717	0.00081
	Mixing of species – Yes	1.0793679	1.0271239– 1.134269	0.00265
	Grazing in areas where wild anmals graze	1.0546803	1.014184 – 1.096792	0.00788
	Presence of ppr	1.0564274	1.00397 - 1.11162	0.035515
SRMV antibodies seropositivity	Farming systems - pastoral	1.154254	1.0464107– 1.273212	0.004281
	Mixing of flocks – Yes	1.060278	1.0091291– 1.136546	0.049095
	Grazing in areas where wild anmals graze	1.077164	1.0031831 – 1.156601	0.040997
	Provision of extra feeds at home - Yes	1.193309	1.0907426 – 1.305521	0.000127
	Presence of CCPP	1.310761	1.00929 - 1.7023	0.04337

(iii) Univariate logistic regression analysis of risk factors associated with *M. capripneumoniae* and SRMV antibodies seropositivity in sheep

In sheep, *M. capripneumoniae* was significantly low in pastoral farming system, and when sick animals were present in the flocks but was significantly high in flocks where SRMV seropositivity was high. Farming systems, introducing new animals in the flock, provisions of extra feeds at home and outbreak in the past 12 months were risk factors significant risk factors for SRMV spread as given in Table 8.

Table 8: Univariate logistic regression analysis for *M. capripneumoniae* and SRMV antibodies seropositivity in sheep

Description	Risk factor	Odds Ratio	95% CI	*p-value*
M. capripneumoniae seropositivity	Farming system -pastoral	0.9434118	0.89574 - 0.9934	0.0284
	Presence of sick animals in the past six months	0.9222472	0.87017 - 0.97744	0.00674
	Presence of ppr	1.035626	0.98509 - 1.0887	0.1713
SRMV antibodies seropositivity	Farming system –pastoral	1.166119	1.03424 – 1.3148	0.0126
	Introducing new animals in the flocks	1.363749	1.0538 - 1.76481	0.019
	Provision of extra feed at home - Yes	1.289054	1.10369 - 1.50555	0.0015
	Presence of sick animals in the past six months	1.090073	0.95115 - 1.2493	0.216
	Introducing new animals in the past 12 months - Yes	1.107089	0.95681 - 1.2809	0.173
	Outbreak in the past 12 months - Yes	1.181876	1.0607 – 1.3169	0.00269
	Mixing of flocks	1.106641	0.97599 - 1.2548	0.115
	Grazing in areas where wild anmals graze	1.084296	0.97185 - 1.2098	0.149
	–Presence of CCPP	1.207612	0.92227 – 1.5812	0.171

(iv) Multivariate logistic regression analysis of risk factors associated with *M. capripneumoniae* and SRMV antibodies seropositivity in sheep

Farming system was the risk factors that influenced occurrence of *M. capripneumoniae* seropositivity in sheep. On the other hand, SRMV was significantly influenced by farming system, provision of extra feeds at home, mixing of flocks and presence of outbreaks in the flocks in the past 12 months as shown in Table 9.

Table 9: Multivariate logistic regression analysis of risk factors associated with *M. capripneumoniae* and SRMV antibodies seropositivity in sheep

Description	Variable	Odds Ratio	95% CI	*p-value*
M. capripneumoniae seropositivity	Farming system - pastoral	0.9310771	0.88363 - 0.98107	0.007885
SRMV antibodies seropositivity	Farming system - pastoral	1.188681	1.05599 - 1.3381	0.00453
	Provision extra feeds at home	1.256354	1.07344 - 1.4704	0.00480
	Mixing of flocks	1.135146	1.00015 - 1.2884	0.05073.
	Outbreak in the past 12 months-Yes	1.161883	1.0396 - 1.2986	0.00865

4.3 Validation of field disease surveillance reporting system accuracy on diseases manifesting with respiratory signs in small ruminants in Tanzania

4.3.1 Molecular detection of the pathogens from samples obtained from goats and sheep

The tentative diagnoses carried out using the FD-1 and FD-2 were confirmed by molecular techniques based on PCR/REA for *M. capripneumoniae* and PCR for other *M. mycoides* cluster members, *P. multocida*, *M. haemolytica* and SRMV. Laboratory results confirmed that some samples were positive for members of the *Mycoplasma mycoides* "cluster" with 548bp were confirmed by PCR (Fig. 5).

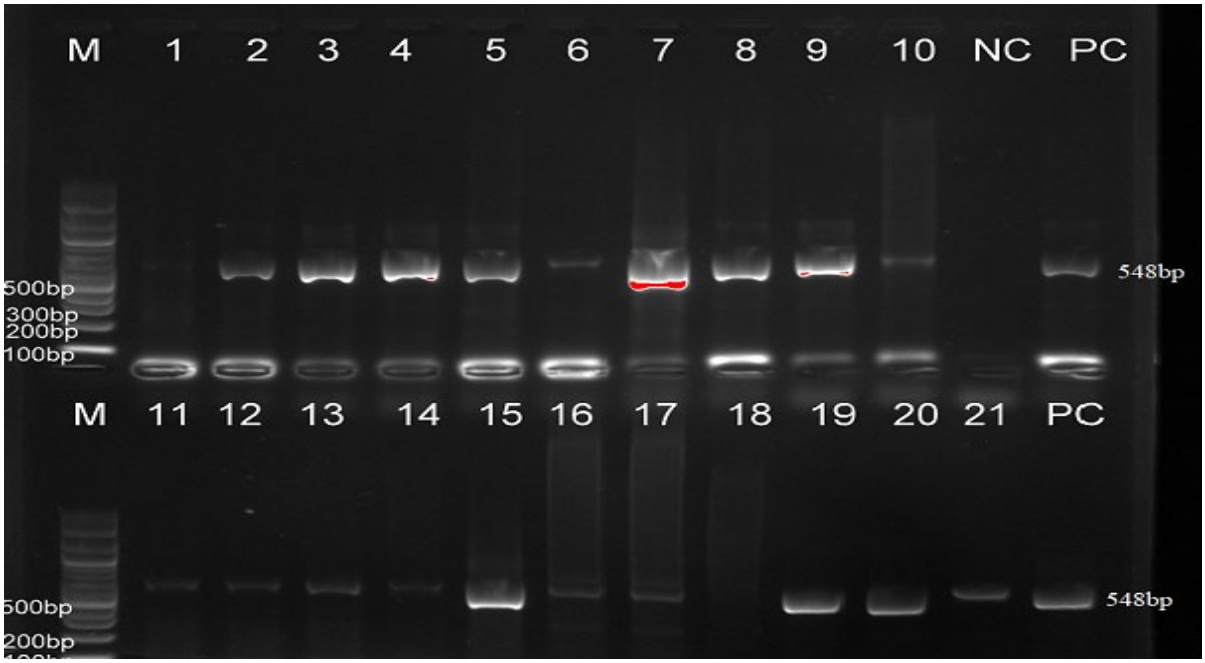

Figure 5: Gel electrophoresis picture showing the 16S rRNA gene (548bp)

First row; M = 100 bp ladder, lanes 1, 6 and 10 = negative samples, lanes 2, 3, 4, 5, 7, 8 and 9 = positive samples, NC = negative control, PC = positive control (*M. capripneumoniae*), Second row; M =100 bp ladder, lanes 11, 1, 13, 14, 16, 17 and 18 = negative samples, lanes 15, 19, 20 and 21 positive samples, PC = positive control (*M. capricolum*).

Presence of *M. caprineumoniae*, the causative agent for CCPP was confirmed in 20.9% (31/148) of the goat cases. A total of 15.1% (2/57) cases of the sheep were confirmed to be positive for *M. capripneumoniae* infection. Presence of two other members of the *M. mycoides* "cluster", that is, *M. capri* and *M. capricolum* were confirmed in 9.5% (14/148) and 6.8% (9/148) of the samples, respectively (Fig. 6 and Fig. 7).

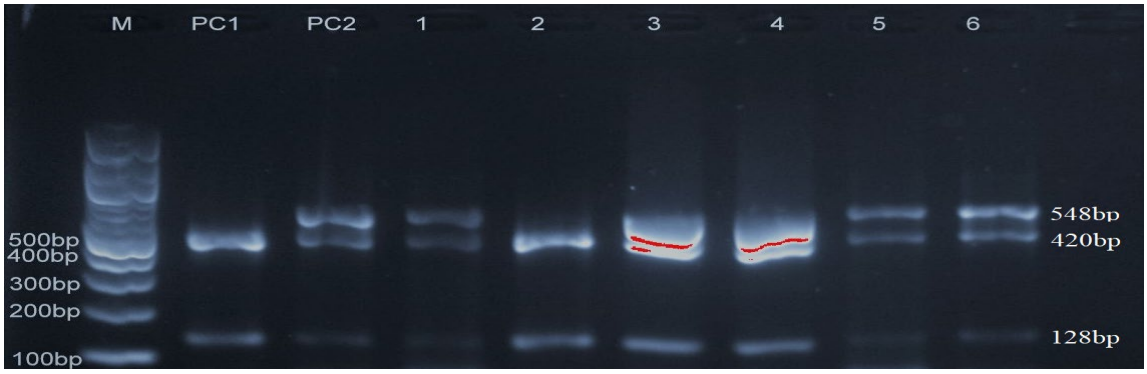

Figure 6: Gel electrophoresis picture showing digested bands of the 16S rRNA gene

PC1=Positive control for *M. capri*; PC2 = Positive control for *M. capripneumoniae,* M=100bp ladder*; M. capripneumoniae* (1, 5, 6) and other members of *M. mycoides* "cluster" (2, 3, 4).

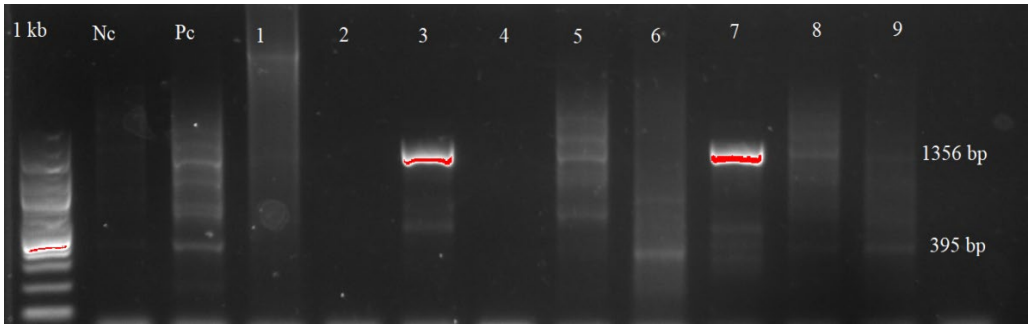

Figure 7: Gel electrophoresis picture showing LppA gene (1356 bp) and CAP-21 gene (395 bp)

PC=Positive controls; NC = Negative control; 1 kb=ladder, *M. capricolum* (3, 5, 7) and *M. capri* (3, 6, 9).

Pneumonic pasteurellosis was confirmed in 39.9% (59/148) and 49.1% (28/57) of goats and sheep cases, respectively whereas, pneumonic mannheimiosis was confirmed in 6.8% (10/148) and 15.8% (9/57) of goats and sheep cases, respectively (Fig. 8).

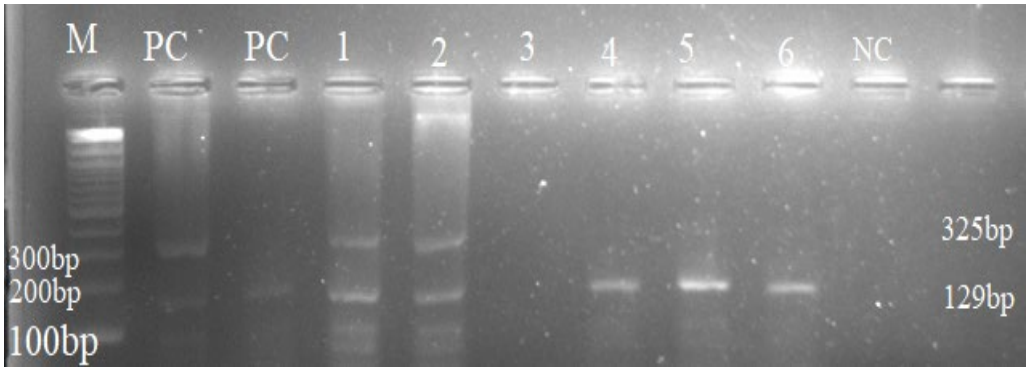

Figure 8: Gel electrophoresis picture showing SSA-1 gene (325bp) and Omp87 gene (129bp)

PC = positive control for *P. multocida* and *Mannheimia hemolytica,* NC = negative control, M=100bp ladder. *M. haemolytica* and *P. multocida* (1, 2), *P. multocida* (4, 5, 6)

Peste des petits ruminants with a nucleoprotein (N) gene with 351bp was confirmed in 25.7% (38/148) and 26.3% (15/57) of goats and sheep cases, respectively (Fig. 9).

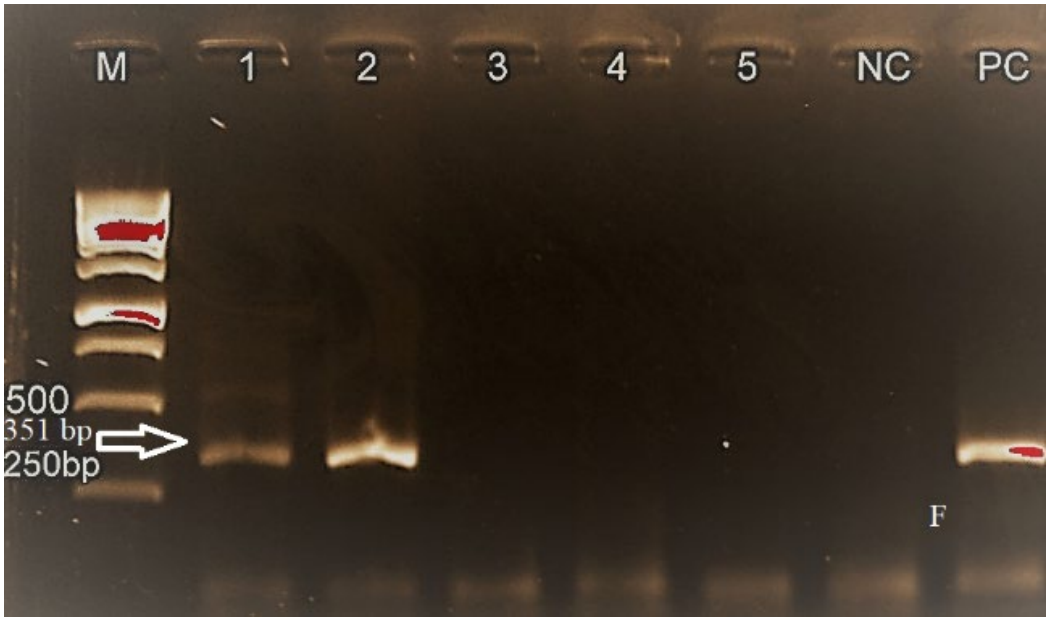

Figure 9: Gel electrophoresis picture showing the Nucleoprotein (N) gene (351bp)

PC = Positive control, NC = Negative control, M = ladder, Samples 1 and 2 were positive with 351 bp nucleoprotein gene

4.3.2 Comparison of the field and laboratory results obtained from goats

The use of field data form one (FD-1) detected higher proportions of both CCPP and PPR compared to FD-2 surveillance forms relative to molecular analysis that was used for confirmation (Table 10). On the other hand, concurrent infections were detected only under the FD-2.

Table 10: Proportions (percentage) of diseases diagnosed based on field and molecular approaches in goats

Diagnosis/Tentative diagnosis	FD-1 (95% CI)[†]	FD-2 (95% CI)[†]	LD (95% CI)[†]
CCPP	45.9 (37.8 – 54.3)	30.4 (23.3 – 38.6)	20.9 (14.9 – 28.6)
Undefined pneumonia	4.1 (1.7 – 9.0)	14.9 (9.7 – 21.9)	-
PPR	50.0 (42.0 – 58.0)	23.0 (16.6 – 30.7)	25.7 (19.0 – 33.6)
CCPP + PPR[§]	NR	19.6 (13.7 – 27.1)	-
PPR + Undefined pneumonia[§]	NR	10.8 (6.5 – 17.2)	-
CCPP + PPR + Undefined pneumonia[§]	NR	0.7 (0.0 – 4.2)	-
PPR + Goat pox[§]	NR	0.7 (0.0 – 4.2)	-
M. capri pneumonia	NR	NR	9.5 (5.4 – 15.7)
M. capricolum pneumonia	NR	NR	6.8 (3.5 – 12.4)
Pneumonic pasteurellosis	NR	NR	33.1 (25.7 – 41.4)
Pneumonic mannheimiosis	NR	NR	6.8 (3.5 – 12.4)

Legend:

[§]Concurrent infections; Not Reported (NR), [†]Confidence interval adjusted for stratification and clustering of cases by specific diseases

4.3.3 Accuracy of FD-1 and FD-2 in diagnosing CCPP and PPR in goats

The FD-2 performed better than FD-1 in diagnosing CCPP where there were *slight* (κ=0.02) agreement between FD-2 and LD but *poor* (κ=-0.09) agreement between FD-1 and LD and, PPR where both FD-1 and FD-2 had a *fair* (κ=0.32) and (κ=0.40) agreement with LD. Field forms two also had higher positive predictive values of 22.2% and 50%; and specificity of 70.1% and 84.5%, respectively as shown in Table 11. However, the sensitivity was low when compared to FD-1 tool.

Table 11: Performance of FD-1 and FD-2 in diagnosing CCPP and PPR in goats

Diseases	Estimations	FD-1 (%)	FD-2 (%)	LD (%)
CCPP	Sensitivity	35.5	32.3	20.9[†]
	Specificity	51.3	70.1	
	Positive predictive value	16.2	22.2	
	Kappa coef.	-0.09	0.02	
PPR	Sensitivity	84.2	44.7	25.7[†]
	Specificity	61.8	84.5	
	Positive predictive value	43.2	50.0	
	Kappa coef.	0.32	0.40	

Legend. [†]Prevalence

4.3.4 Zonal variations in the diagnoses of CCPP and PPR based on the three approaches in goats

The three detection tools did not diagnose CCPP in the lake zone whereas, the proportions of PPR detection was almost similar in the northern zone. Based on the overall performance, FD-2 detected higher proportion than FD-1 for both CCPP and PPR when compared to PCR results as shown in Table 12.

Table 12: Zonal variations on the detection of CCPP and PPR in goats based on the three approaches

Surveillance zones	Contagious caprine pleuropneumonia			Peste des petits ruminants		
	FD-1 (95% CI)[ǂ]	FD-2 (95% CI)[ǂ]	LD (95% CI)[ǂ]	FD-1 (95% CI)[ǂ]	FD-2 (95% CI)[ǂ]	LD (95% CI)[ǂ]
Central	60.0 (32.9 – 82.5)	86.7 (58.4 – 97.7)	19.4 (8.1 – 38.1)	13.3 (2.3 – 41.6)	13.3 (2.3 – 41.6)	2.6 (0.1 – 15.4)
Eastern	0.0	0.0	16.1 (6.1 – 34.5)	100	66.7 (35.4 – 88.7)	13.2 (4.9 – 28.9)
Lake	0.0	0.0	0.0	100	0.0	21.1 (10.1 – 37.8)
Northern	69.7 (57.0 – 80.1)	25.8 (16.1 – 38.2)	16.1 (6.1 – 34.5)	30.3 (19.9 – 43.0)	36.4 (25.1 – 49.2)	34.2 (20.1 – 51.4)
Southern highlands	33.3 (19.6 – 50.3)	38.5 (23.8 – 55.3)	48.4 (30.6 – 66.6)	61.5 (44.7 – 76.2)	0.0	28.9 (16.0 – 46.1)
Overall	45.9 (37.8 – 54.3)	30.4 (23.3 – 38.6)	20.9 (14.9 – 28.6)	50.0 (42.0 – 58.0)	23.0 (16.6 – 30.7)	25.8 (19.0 – 33.6)

Legend: [ǂ]Confidence interval adjusted for stratification by surveillance zones and clustering of cases by specific diseases

4.3.5 Accuracy of FD-1 and FD-2 in diagnosing CCPP in goats in different zones

In the lake zone both FD-1 and FD-2 had an *almost perfect* (κ=1.0) agreement with LD in diagnosing CCPP. The performance of FD-2 was better than FD-1 in all zones, with moderate (κ=0.45) and (κ=0.55) in eastern and northern zones respectively as given in Table 13.

Table 13: Accuracy of FD-1 and FD-2 in diagnosing contagious caprine pleuropneumonia in goats in different surveillance zones

Surveillance zones	Contagious caprine pleuropneumonia					
	FD-1			FD-2		
	Sensitivity (%)	Specificity (%)	*Kappa coef.*	Sensitivity (%)	Specificity (%)	*Kappa coef.*
Central	33.3	22.2	-0.41	100.0	22.2	0.19
Eastern	0.0	100.0	0.0	0.0	100.0	0.45
Lake	0.0	100.0	1.0	0.0	100.0	1.0
Northern	100.0	32.8	0.07	0.0	72.1	0.55
Southern highlands	26.7	62.5	-0.11	26.7	54.2	0.19

4.3.6 Accuracy of FD-1 and FD-2 in diagnosis of PPR in goats in different surveillance zones

In diagnosing PPR, both FD-1 and FD-2 had Cohen's kappa values ranging from 0.00 to 0.93. In the central zone, northern zone and southern highland zone FD-2 agreement to LD increased to *almost perfect* (κ=0.93), *moderate* (κ=0.53) and *substantial* (κ=0.61), respectively as shown in Table 14.

Table 14: Accuracy of FD-1 and FD-2 in diagnosing peste des petits ruminants in goats in different surveillance zones

Surveillance zones	Peste des petits ruminants					
	FD-1			FD-2		
	Sensitivity (%)	Specificity (%)	*Kappa coef.*	Sensitivity (%)	Specificity (%)	*Kappa coef.*
Central zone	100.0	92.9	0.63	100.0	92.9	0.93
Eastern zone	41.7	0.0	0.00	92.9	42.9	0.21
Lake zone	53.3	0.0	0.00	0.0	100.0	0.00
Northern zone	61.5	77.4	0.32	92.3	77.4	0.53
Southern highlands zone	90.9	50.0	0.30	0.0	100.0	0.61

4.3.7 Comparison of the field and laboratory results obtained from sheep

Field data forms one (FD-1) and FD-2 detected higher proportions of PPR as compared to the laboratory molecular diagnosis. Field data form one (FD-1) had the highest proportions of *M. capripneumoniae* pneumonia as compared to FD-2 relative to LD obtained results. In both scenarios, FD-2 performed better than FD-1 in detecting PPR but none detected pasteurellosis with exception of LD as shown in Table 15.

Table 15: Proportions (percentage) of diseases diagnosed based on field and molecular approaches in sheep

Diseases diagnosed	FD-1 (95% CI)[ŧ]	FD-2 (95% CI)[ŧ]	LD (95% CI)[ŧ]
M. capripneumoniae pneumoniae	14.0 (6.7 – 26.3)	5.3 (1.4 – 15.5)	3.5 (0.6 – 13.2)
Undefined pneumonia	-	35.1 (23.2 – 48.9)	-
Peste des petits ruminants (PPR)	86.0 (73.7 – 93.3)	45.6 (32.6 – 59.2)	26.3 (19.0 – 33.6)
M. capripneumoniae pneumoniae + PPR[§]	NR	7.0 (2.3 - 17.8)	-
PPR + Undefined pneumonia [§]	NR	7.0 (2.3 - 17.8)	-
Pneumonic pasteurellosis	NR	NR	31.6 (20.3 – 45.4)
Pneumonic mannheimiosis	NR	NR	15.8 (7.9 – 28.4)

Legend: [§]Concurrent diseases; Not reported (NR) & [ŧ]Confidence interval adjusted for stratification and clustering of cases by specific diseases

4.3.8 Accuracy of FD-1 and FD-2 in diagnosing *M. capripneumoniae* pneumonia and PPR in sheep

Field data forms two (FD-2) and FD-1 performed better in the diagnosis of PPR than *M. capripneumoniae* pneumonia, The FD-1 and FD-2 had *slight* (κ=0.06) agreement with LD in the diagnosis of PPR with positive predictive values of 23.1% and 26.3%, respectively. But both FD-1 (κ=-0.06) and FD-2 (κ=-0.04) had *poor* agreement with LD in the diagnosis of *M. capripneumoniae* pneumonia However, in the diagnosis of *M. capripneumoniae* pneumonia, despite the fact that FD-1 and FD-2 had *poor* agreement with LD, FD-2 had a better performance compared to FD-1 as shown in Table 16.

Table 16: Accuracy of FD-1 and FD-2 in diagnosing *M. capripneumoniae* pneumonia and peste des petits ruminants in sheep

Diseases	Sensitivity/Specificity	FD-1 (%)	FD-2 (%)	LD (%)
CCPP	Sensitivity	0.0	0.0	3.5[†]
	Specificity	85.5	94.5	
	Positive predictive value	0.0	0.0	
	Kappa coef.	-0.06	-0.04	
PPR	Sensitivity	100.0	40.0	26.3[†]
	Specificity	16.0	52.4	
	Positive predictive value	23.1	26.3	
	Kappa coef.	0.06	0.06	

Legend. [†]True prevalence

4.3.9 Zonal variations in the diagnosis of *M. capripneumoniae* pneumonia and PPR based on the three approaches in sheep

As it was in FD-1 and FD-2, LD also confirmed that there was no *M. capripneumoniae* pneumonia in the lake zone. Although FD-1 reported 62.5% of *M. capripneumoniae* pneumonia in the northern zone, FD-2 and LD results were negative as shown in Table 17. In the overall proportions FD-2 performed better than FD-1 in diagnosing both *M. capripneumoniae* pneumonia and PPR as given in Table 17.

Table 17: Zonal variations on the detection of *M. capripneumoniae* pneumonia and PPR in sheep based on the three diagnostic tools

Surveillance zones	*M. capripneumoniae* pneumonia			Peste des petits ruminants		
	FD-1(95% CI)[†]	FD-2 (95% CI)[†]	LD (95% CI)[†]	FD-1 (95% CI)[†]	FD-2 (95% CI)[†]	LD (95% CI)[†]
Eastern	25.0 (4.5 – 64.4)	66.7 (12.5 – 98.2)	0.0	30.6 (18.7 – 45.6)	0.0	26.7 (8.9 – 55.2)
Lake	0.0	0.0	0.0	8.2 (2.6 – 20.5)	0.0	13.3 (2.4 – 41.6)
Northern	62.5 (25.9 – 89.8)	0.0	0.0	53.1 (38.4 – 67.2)	100	40.0 (17.5 – 67.1)
Southern highlands	12.5 (0.7 – 53.3)	33.3 (1.8 – 87.5)	100	8.2 (2.6 – 20.5)	0.0	20.0 (5.3 – 48.6)
Overall	14.0 (6.7 – 26.3)	5.3 (1.4 – 15.5)	3.5 (0.6 – 13.2)	86.0 (73.6 – 93.3)	45.6 (32.6 – 59.2)	26.3 (15.9 – 39.9)

Legend: [†]Confidence interval adjusted for stratification by administrative zones and clustering of cases by specific diseases

4.3.10 Accuracy of FD-1 and FD-2 in diagnosing *M. capripneumoniae pneumonia* in sheep in different surveillance zones

The agreement between FD-1 and FD-2 over LD ranged from 0.0 -1.0 except in the southern highland zone where FD-1 had a *poor* (κ=-0.36) agreement but improved when FD-2 was used. *An almost perfect* agreement was observed between FD-1 and FD-2 (κ=1.0) in the lake zone as shown in Table 18.

Table 18: Accuracy of FD-1 and FD-2 in diagnosing *M. capripneumoniae* pneumonia in sheep in different surveillance zones

Surveillance zones	*M. capripneumoniae* pneumonia					
	FD-1			FD-2		
	Sensitivity (%)	Specificity (%)	*Kappa coef.*	Sensitivity (%)	Specificity (%)	*Kappa coef.*
Eastern	0.0	88.2	0.0	0.0	88.2	0.33
Lake	0.0	100.0	1.0	0.0	100.0	1.0
Northern	0.0	83.9	0.0	0.0	100.0	1.0
Southern highlands	0.0	66.7	-0.36	0.0	66.7	0.41

4.3.11 Accuracy of FD-1 and FD-2 in diagnosing PPR in sheep in different zones

The accuracy of FD-1 in diagnosing PPR was *poor* to *fair* whereas, FD-2 was fair to moderate agreements in all zones as shown in Table 19.

Table 19: Performance of FD-1 and FD-2 in diagnosing PPR in sheep in different surveillance zones

Surveillance zones	Peste des petits ruminants					
	FD-1			FD-2		
	Sensitivity (%)	Specificity (%)	*Kappa coef.*	Sensitivity (%)	Specificity (%)	*Kappa coef.*
Eastern	100.0	13.3	0.08	27.6	100.0	0.57
Lake	100.0	0.0	0.0	43.0	100.0	0.32
Northern	100.0	16.7	0.09	100.0	20.0	0.09
Southern highlands	100.0	33.3	0.21	35.0	100.0	0.55

4.4 Determination of the concurrent infections in outbreaks of diseases which manifest with respiratory signs in small ruminants in Tanzania

4.4.1 Molecular analysis of samples collected from small ruminants during disease outbreaks

Out of 205 cases that were examined, 72.2% (n=205) were goats and 27.8% sheep (n=205). Of the goat's cases 70.9% were live animals and 29.1% cadavers (n=148). For sheep, 68.4% were live animals and 31.6% were cadavers (n=57) as shown in Table 20.

Table 20: Proportions of affected animals by respective diseases among those examined during outbreaks presenting with respiratory signs

Diagnosis	Goats (*n =148*)		Sheep (*n=57*)		Total (*n=205*)
	Live animals (*n=105*)	Dead animals (*n=43*)	Live animals (*n=39*)	Dead animals (*n=18*)	
Contagious caprine pleuropneumonia	14.3 (15)	37.2 (16)	15.1 (2)	0.0 (0)	16.1% (33)
M. capricolum pneumonia	1 (1)	18.6 (8)	0.0 (0)	0.0 (0)	4.4% (9)
M. capri pneumonia	5.7 (6)	18 (8)	0.0 (0)	0.0 (0)	6.8% (14)
Pneumonic pasteurellosis	44.8 (47)	27.9 (12)	53.8 (21)	38.9 (7)	42.4% (87)
Pneumonic mannheimiosis	2.9 (3)	16.3 (7)	15.4 (6)	16.7 (3)	9.3% (19)
Peste des petits ruminants	29.5 (31)	16.3 (7)	28.2 (11)	22.2 (4)	25.9% (53)

4.4.2 Concurrent infections in outbreaks of diseases presenting with respiratory signs in goats

Concurrent infections were confirmed in 79.1% (117/148) of all samples of goats tested in the laboratory. Pathogens which were involved in many concurrent infections were *P. multocida* which was detected in 47.0% (55/117). The SRMV was detected in 41.9% (49/117) and *M. capripneumoniae* in 40.2% (47/117). The highest proportion of concurrent diseases were *M. capripneumoniae* and SRMV, 11.1% (n=117), followed by *M. capricolum* and *P. multocida*, and, *P. multocida* and SRMV both in 9.4% (n=117) of the examined cases as shown in Table 21.

Table 21: Concurrent infections presenting with respiratory signs in goats

Concurrent infections	Frequency (*n=117*)	Percentage (%)
M. capripneumoniae and *M. capri*	9	7.7
M. capripneumoniae and *M. capricolum*	8	6.8
M. capricolum and P. multocida	11	9.4
M. capricolum and M. haemolytica	5	4.3
M. capripneumoniae and SRMV	13	11.1
M. capri and M. capricolum	6	5.1
M. capri and P. multocida	8	6.8
M. capri and SRMV	5	4.3
P. multocida and *M. haemolytica*	5	4.3
P. multocida and SRMV	11	9.4
M. haemolytica and SRMV	4	3.4
M. capripneumoniae, *M. capri* and *M. capricolum*	4	3.4
M. capripneumoniae, *M. capri* and *P. multocida*	3	2.6
M. capripneumoniae and *M. capri* pneumonia and SRMV	4	3.4
M. capripneumoniae and *M. capricolum* and *P. multocida*	2	1.7
M. capripneumoniae and *M. capricolum* and SRMV	1	0.9
M. capripneumoniae, *P. multocida* and *M. haemolytica*	1	0.9
M. capripneumoniae, *P. multocida* and SRMV	2	1.7
M. capri, *M. capricolum* and *P. multocida*	6	5.1
M. capri, *M. capricolum* and SRMV	3	2.6
M. capri, *P. multocida* and SRMV	2	1.7
M. capricolum, P. multocida and SRMV	2	1.7
M. capri, M. capricolum, P. multocida and SRMV	2	1.7

4.4.3 Clinical signs and postmortem lesions observed in concurrent infections in goats

In most of the concurrent infections that involved *M. capripneumoniae* the major clinical signs were difficult breathing, reluctance to move and emaciation, whereas hydrothorax, lung attachment to the chest wall and unilateral or asymmetric lung inflammation (Fig. 10) were the most frequently postmortem lesions. However, together with clinical signs such as nasal discharges, coughing and difficult breathing, and postmortem lesions such as consolidated lungs and lung attachment to the chest wall, when SRMV was involved, clinical signs and postmortem lesions that are associated with the digestive system like anorexia and diarrhea, and vesicular lesions on the gums were also noted as shown in Table 22.

Table 22: Clinical signs and postmortem lesions associated with concurrent infections in goats

Concurrent detection	Cases (%)	Clinical signs	Cases (%)	Postmortem lesions
M. capripneumoniae, *M. capri*	66.7	Coughing, nasal discharges, difficult breathing, reluctance to move, emaciation	77.8	Consolidated lungs, hydrothorax, lung attachment to the chest wall, unilateral lung inflammation
M. capripneumoniae, *M. capri*, *M. capricolum*, *P. multocida*, SRMV	100	Anorexia, coughing, reluctance to move, difficult breathing, diarrhea, emaciation	100	Consolidated lungs, hydrothorax, vesicular lesions on the gums, asymmetric lung inflammation, lung attachment to the chest wall
M. capripneumoniae, *P. multocida*	100	Coughing, reluctance to move, nasal discharges, emaciation	100	Lung attachment to the chest wall, hydrothorax, unilateral lung inflammation
M. capripneumoniae, SRMV	75	Anorexia, diarrhea, coughing, nasal discharges, difficult breathing, reluctance to move, emaciation	62.5	Vesicular lesions on the gums, lung attachment to the chest wall, unilateral lung inflammation
M. capri, *P. multocida*	87.5	Coughing, nasal discharges, difficult breathing	62.5	Consolidated lungs, Lung attachment to the chest wall
P. multocida, *M. haemolytica*	60	Anorexia, nasal discharges	80	Tracheal froth, soft hemorrhagic regional lymph nodes
P. multocida, SRMV	81.1	Anorexia, diarrhea, nasal discharges	54.5	Tracheal froth , vesicular lesions on the gums

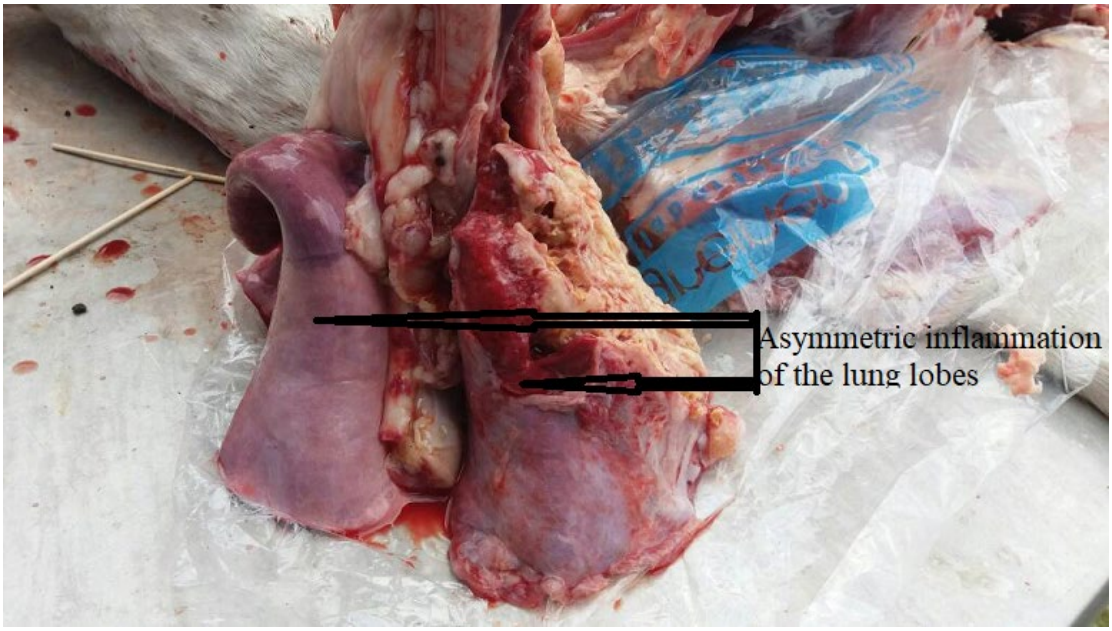

Figure 10: Asymmetrical inflamed goat lung lobes with concurrent infection involving CCPP

The goat Lab ID case no. Mond.1 which tested positive to *M. capripneumoniae*, *M. capri*, *M. capricolum*, *P. multocida* and SRMV

4.4.4 Concurrent infections in outbreaks presenting with respiratory signs in sheep

In sheep, concurrent infections were diagnosed in 28.1% (16/57) of the samples tested in the laboratory. Small ruminants morbillivirus was the pathogen highly detected in concurrent infections with other diseases. The highest number of cases with concurrent infections was 31.3% (n=16) which involved *P. multocida* and SRMV whereas, the lowest was 12.5% (n=16) which involved *M. capripneumoniae* and SRMV and also, *M. haemolytica* and SRMV as shown in Table 23.

Table 23: Concurrent infections presenting with respiratory signs in sheep

Concurrent detection	Frequency (*n=16*)	Percentage (%)
M. capripneumoniae and SRMV	2	12.5
P. multocida and *M. haemolytica*	4	25.0
P. multocida and SRMV	5	31.3
M. haemolytica and SRMV	2	12.5
P. Multocida, *M. haemolytica* and SRMV	3	18.8

4.4.5 Clinical signs and postmortem lesions observed in concurrent infections in sheep

In sheep, SRMV was involved in most of the concurrent detection with anorexia; diarrhea and nasal discharges being the most frequently associated clinical signs. When SRMV and *M. capripneumoniae* were concurrently infecting sheep, the lesions observed were vesicular lesions on the gums and hemorrhages on the small intestines as shown in Table 24.

Table 24: Clinical signs and postmortem lesions associated with concurrent infections in sheep

Concurrent detections	Cases (%)	Clinical signs	Cases (%)	Postmortem lesions
P. multocida, SRMV	80	Anorexia, nasal discharges diarrhea	80	Consolidated lungs, tracheal froth, congested nasal cavity
P. multocida, *M. haemolytica*, SRMV	100	Nasal discharges, diarrhea, anorexia	66.7	Consolidated lungs, tracheal froth, congested nasal cavity
M. capripneumoniae, SRMV	100	Diarrhea, coughing, nasal discharges, ocular discharges, difficult breathing, reluctance to move	100	Vesicular lesions on the gums and hemorrhages on the small intestines.
M. haemolytica, *P. multocida*	75	Anorexia, coughing, nasal discharges,	100	Increased pericardial fluid
M. haemolytica, SRMV	100	Nasal discharges, diarrhea, anorexia	100	Tracheal froth, congested nasal cavity

4.5 Discussion

This study reports on the presence of diseases presenting with respiratory signs in small ruminants in Tanzania. These diseases cause serious economic losses in small ruminants as they persist in field, despite the efforts made to control them, these findings are supported by previous studies by Kgotlele *et al.* (2018). Concurrent infections, misdiagnosis and partial diagnosis are reported in this study as the major setbacks in setting up proper control strategies, the major problem being lack of facilities and expertise in rural settings and the need to rely on visual clinical and pathological examinations in diagnosis.

In objective one, risk factors for *M. capripneumoniae* spread were determined, and the results obtained showed that farming system negatively correlated with *M. capripneumoniae* seropositivity whereas, mixing of species, grazing in areas where wild anmals graze, presence of PPR perpetuated the spread of *M. capripneumoniae* in goats. Results also showed that SRMV spread was perpetuated by farming systems, mixing of flocks, grazing in areas where wild anmals graze, provision of extra feeds at home and presence of CCPP. Furthermore, risk factors for spread of *M. capripneumoniae* in sheep were determined and results showed that pastoral farming negatively correlated with spread of *M. capripneumoniae* in sheep. In determining risk factors for spread of SRMV in sheep, farming system, provision extra feeds at home, mixing of flocks and outbreak in the past 12 months positively correlated with SRMV seropositivity in sheep.

Mixing of species (caprine and ovine) was a significant risk factor associated with *M. capripneumoniae* antibodies seropositivity (OR=1.0793679) in goats this may be due to the fact that sheep are known to harbor *M. capripneumoniae* (Bölske *et al.,* 1995). In goats, grazing in areas where wild animals graze (OR=1.0546803), was another significant risk factors for *M. capripneumoniae* antibodies seropositivity, this finding indicates the possibility of *M. capripneumoniae* being a significant risk in wild life conservation. *Mycoplasma capripneumoniae* presence in wild life was also reported in Qatar (Arif *et al.,* 2007) and in endangered Markhors (*Capra falconeri*) in Tajikistan (Ostrowski *et al.*, 2011). Presence of PPR (OR=1.0564274) also was positively correlated with the spread of *M. capripneumoniae*, the results are in line with the results on concurrent infection reported by Kgotlele *et al.* (2018). In this study, *M. capripneumoniae* in goats had lower odds in pastoral farming systems (OR=0.91073332), this could be due to lower numbers of goats in pastoral settings where sheep are kept in large numbers. The lower odds reported in this study are contrally to

the previous study by Teshome *et al.* (2018) who reported higher odds in Borana pastoral community in Ethiopia. Mixing of species (caprine and ovine) was a significant risk factor associated with *M. capripneumoniae* antibodies seropositivity (OR=1.0793679) in goats this may be due to the fact that sheep are known to harbor *M. capripneumoniae* (Bölske *et al.,* 1995). In goats, grazing in areas where wild animals graze (OR=1.0546803) like in Mbarali, areas close to Ruaha National Park, was another significant risk factors for *M. capripneumoniae* antibodies seropositivity, this finding indicates the possibility of *M. capripneumoniae* being a significant risk in wildlife conservation. *Mycoplasma capripneumoniae* presence in wildlife was also reported in Qatar (Arif *et al.,* 2007) and in endangered Markhors (*Capra falconeri*) in Tajikistan (Ostrowski *et al.*, 2011). Presence of PPR (OR=1.0564274) also was positively correlated with the spread of *M. capripneumoniae*, the results are in line with the results on concurrent infection reported by Kgotlele, *et al.* (2018). In this study, *M. capripneumoniae* in goats had lower odds in pastoral farming systems (OR=0.91073332), this could be due to lower numbers of goats in pastoral settings where sheep are kept in large numbers. The lower odds reported in this study are contrally to the previous study by Teshome *et al.* (2018) who reported higher odds in Borana pastoral community in Ethiopia. Furthermore, the results pastoral farming system (OR=1.154254), mixing of flocks (OR=1.060278) and provision of extra feeds at home (OR=1.193309), were risk factors associated with SRMV seropositivity in goats. These findings are in agreement with the findings reported in the previous studies, that congregation of animals from different flocks at grazing and watering points increased the chances of occurrence of both diseases (Lefèvre *et al.*, 1987; Ozdemir *et al.*, 2005; Kusiluka *et al.*, 2007). Grazing in areas where wild anmals graze (OR=1.077164) like in Babati close to both Tarangire and Lake Manyara National Parks was another risk factor that positively correlated with SRMV antibody seropositivity in goats indicating the serious threat of the disease in the livestock-wild life interphase. The results are in line with the previous study by Aguilar *et al.* (2020) who reported on the epidemiological linkage between epizootic cycles in livestock and exposure in wildlife. The results from this further indicate the importance of domestic-wildlife interaction in the spread, control and eradication of SRMV (Fine *et al.*, 2020). The spillover of SRMV from sheep to wildlife in Serengeti ecosystem (Mahapatra *et al.*, 2015), is another evidence of the importance of the role of wildlife-livestock interface in SRMV control, which may include controlling legal or illegal movement of livestock in the wildlife territories (Marashi *et al.*, 2017). Presence of CCPP (OR=1.310761) on the other hand positively

correlated with SRMV spread in goats, and this result is in line with the results on concurrent infections reported by Kgotlele *et al.* (2018).

The results from this study shows that, in sheep pastoral farming system (OR=0.9310771) risk factor negatively correlated with *M. capripneumoniae* antibody seropositivity. This could be due to the fact that in pastoral farming systems, there were large numbers of sheep. Sheep are not natural hosts of *M. capripneumoniae* (Bölske *et al.*, 1995) and the fact that in pastoral farming system the animals are kept in open areas (Kusiluka *et al.*, 1998). Despite the fact that SRMV affects both goats and sheep (Baazizi *et al.*, 2017), this study reports a higher infection rate in sheep contrary to the previous study by Balamurugan *et al.* (2014), who reported higher prevalence in goats and Agga *et al.* (2019) who reported that there is no significant difference between species. These variations in SRMV infection in different species could be due to differences in study designs, samples collected and status of the animals. Pastoral farming system (OR=1.188681) on the other hand had a positive correlation with SRMV antibody seropositivity in sheep, the results are supported by the findings reported by Herzog *et al.* (2019), who reported higher seropositivity of PPR across goats, sheep and cattle species in pastoral farming systems in northern Tanzania. Provision of extra feeds at home (OR=1.256354) which included farm left overs perpetuated the spread of SRMV, this could be due to the fact that this increased congregation of the animals, the factor that was also reported in previous studies (Lefèvre *et al.*, 1987; Ozdemir *et al.*, 2005; Kusiluka *et al.*, 2007). Mixing of flocks (OR=1.135146) from different areas with in endemic areas increased the risk of coming incontact between sick and healthy animals, this risk factor had a positive correlation with SRMV seropositivity in sheep. The results obtained in this study are in line with the previous study by Herzog *et al.* (2019). Presence of outbreak in the past 12 months (OR also positively correlated with SRMV in sheep, history of previous infections to persist and spread have also been discussed in the review by Idoga *et al.* (2020).

In objective two, the field results obtained from visual examination of the clinical signs and pathological lesion with the aid on FD-1 and FD-2 were validated using the results from a gold standard test. Visual examinations in field diagnosis carried out in this study are also suggested by FAO (http://www.fao.org/3/x3331e/X3331E01.htm). Generally, the laboratory results confirmed presence of *M. capripneumoniae*, *M. capri*, *M capricolum*, *P. multocida*, *M. haemolytica* and SRMV in various samples collected from different zones. The results are in line with the previous studies by Kusiluka *et al.* (2000) for *M. capripneumoniae* and

Kgotlele *et al.* (2018) for SRMV and *P. multocida*. *M. capri*, *M. capricolum* and *M. haemolytica* were confirmed using molecular techniques for the first time in small ruminants. In comparing the performance of FD-1 and FD-2 when the agreements of each of them to LD were evaluated, FD-2 had a better performance as compared to FD-1. However, the performance of both FD-1 and FD-2 were inferior to the LD as anticipated (Ebel *et al.*, 2016). Results showed that, the accuracy of FD-2 in diagnosing CCPP in goats was better than FD-1, whereas, both had a similar accuracy when diagnosing PPR in goats. In sheep, both FD-1 and FD-2 had a better accuracy when diagnosing PPR than when diagnosing M capripneumoniae. FD-1 and FD-2 has a similar accuracy when diagnosing PPR and CCPP. Furthermore, the variations in the performance of both FD-1 and FD-2 in different zones were noted.

In evaluating the performance of field diagnosis of CCPP in goats, FD-1 had *poor* agreement compared to *slight* in FD-2 when LD was used as gold standard. There was improvement in the results when FD-2 was used and this could be attributed to the judgments reached following presence of listed clinical signs and postmortem features in FD-2. In the diagnosis of CCPP, accuracy was enhanced by postmortem, despite the fact that clinical signs can also be suggestive (Teshome *et al.*, 2018). Variations in the performance of FD-1 and FD-2 were observed across the administrative zones, in the central and southern highland zones where the performance increased from *poor* in FD-1 to *slight* in FD-2. The agreement increased from *slight* to *moderate* in FD-1 and FD-2 respectively, when compared to LD in the northern zone whereas, in the eastern and lake zones both FD-1 and FD-2 had an *almost perfect* agreement to LD. The better performance of FD-2 in all zones may further be due to variations attributed to the knowledge and experience of the Livestock Field Officers (LFO's) and District Veterinary Officers (DVO'S) in the respective zones and farming systems as previously also, reported by Morgan *et al.* (2014).

In the diagnosis of PPR in goats, the overall performance of both FD-1 and FD-2 was *fair* (κ*=0.32, 0.4*). Despite the increase in the Kappa value, the class remained the same indicating effect of the changes made in FD-2 did not bring a big change as it was in the diagnosis of CCPP. On the other hand, the results showed that PPR was generally more correctly diagnosed than CCPP irrespective of field forms used. This could be due to the fact that, PPR has been reported more than CCPP and presence of some obvious lesions such as vesicular lesions, intestinal haemorrhages and diarrhea. In addition, knowledge of farmers about the

disease following experience during outbreaks may enhance detection (Kgotlele *et al.*, 2014b; Torsson *et al.*, 2017). In the same way, variations across the administrative zones were observed and associated with the knowledge and experience of the Livestock Field Officers (LFO's) and District Veterinary Officers (DVO'S) in the respective zones and farming systems as previously also, reported by Morgan *et al.* (2014). For example, in the eastern and lake zone FD-1 had a *slight* agreement with LD and the agreement remained the same in lake zone but improved to *fair* in the eastern zone, this may be due to the fact that in eastern zone there were previous reports of PPR outbreaks (Kgotlele *et al.*, 2014b) and it was easy for the diagnosticians to link the clinical signs. A similar trend was observed in the northern, central and southern highlands zone where PPR was also reported in previous studies (Torsson *et al.*, 2017) and the performance improved from *fair* to *moderate*, *fair* to *substantial* and *substantial* to *almost perfect* when FD-1 and FD-2 were compared to LD, respectively.

This study has also revealed that, it was difficult to diagnose *M. capripneumoniae* infection in sheep basing on clinical and postmortem lesions, probably due to the fact that sheep are not natural hosts of *Mycoplasma capricolum* subsp *capripneumoniae* the causative agent of CCPP (Bölske *et al.,* 1995). This study has shown that, generally FD-1 and FD-2 had *poor* (*κ= -0.06; -0.04*) agreement with LD, respectively. Diagnosis accuracy and performance of FD-1 and FD-2 varied across the administrative zones, with the performance in lake zone having an *almost perfect* for both FD-1 and FD-2 in diagnosing PPR. In the northern zone the diagnosticians were able to follow and scrutinize the clinical signs and postmortem lesions to differentiate PPR from CCPP or other mycoplasmas and pasteurella infections because PPR has been reported from the zone in the previous studies (Mekuria *et al.*, 2008; Alemneh & Tewodros, 2017; Torsson *et al.*, 2017), this resulted in improvement in diagnosis from *slight* in FD-1 to *almost perfect* in FD-2. Similar improvement was observed in the southern highland zone where the performance improved from *poo*r in FD-1 to *moderate* in FD-2 as PPR and CCPP were previously reported in the area (Kgotlele *et al.*, 2018). In this study, it was also revealed that sheep had higher proportion of PPR than goats, the findings which are comparable to those reported by Balamurugan *et al.* (2012). However, the overall field diagnosis was *slight* for both FD-1 and FD-2, (*κ=0.06*) when compared to LD. However, major improvement was observed in the southern highland where FD-1 had *fair* but improved to *moderate*, and the eastern zone had *slight* (FD-1) to *Moderate* (FD-2).

Zonal variations may be attributed to the local understanding and awareness of the diseases common in the area (Swai & Neselle, 2010), the list of probable clinical signs and postmortem lesions relevant to diseases presenting with respiratory signs that prompted field personnel to observe, recall and link with the check list in FD-2. Therefore, the observed variations may call for further improvement of the FD2, continued professional development, and enhanced laboratory diagnosis during outbreaks to cub the disease and effective communication for timely response.

In objective three, determination of the concurrent infections in the outbreaks of diseases presenting with respiratory signs was carried out. The results showed that, in goats, 79.1% of the samples had concurrent infections. The pathogens that were commonly detected in most of the concurrent infections in goats were *P. multocida*, SRMV and *M. capripneumoniae* in that order from highest to lowest. Highest proportions of co-occurrence in goats was between *M. capripneumoniae* and SRMV, *M. capricolum* and *P. multocida* and *P. multocida* and SRMV from highest to lowest in that order. The results also showed that concurrent infections involving *M. capripneumoniae* were accompanied by difficult breathing, reluctance to move, standing with extended elbow and emaciation. *Mycoplasma capripneumoniae* concurrent infections also had hydrothorax, lung attachment to the chest wall and unilateral lung inflammation. In one of the cases, there was an asymmetric lung inflammation. In all cases where SRMV was involved, clinical signs and postmortem lesions involving the digestive system were observed accompanying the normal respiratory signs like difficult breathing, nasal discharges and coughing, and postmortem lesions like consolidated lungs and lungs attachment to the chest wall. The results showed that, in sheep, 28.1% of the samples had concurrent infections. The pathogen that was involved in many concurrent infections in sheep was SRMV. Highest proportions of co-occurrence in sheep was between *P. multocida* and SRMV whereas, the lowest was between *M. haemolytica* and SRMV. Results showed that concurrent infections involving SRMV were accompanied by anorexia, diarrhea and nasal discharges whereas, the major postmortem lesions were vesicular lesions in the gums and haemorrhagic intestines.

The classic respiratory disease (CCPP) is caused by *M. capripneumoniae,* and in this study most of the cases were accompanied by other pathogens including, other mycoplasmas like *M. capri* and *M. capricolum*. Concurrent infections of *M. capripneumoniae* and other mycoplasmas were also reported in the previous studies by Kusiluka *et al.* (2000) who

reported *M. capripneumoniae* occurring together with *Mycoplasma mycoides* subspecies *mycoides* small colony type. Furthermore, in this study *M. capripneumoniae* has been reported to co-infect with *P. multocida* and *M. haemolytica* in goats, the results are in line with the study reported by Settypalli *et al.* (2016) and Kgotlele *et al.* (2018). These concurrent infections result in partial and misdiagnosis. Presence of SRMV in any of the co-occurrence that culminates in presenting respiratory signs complicated the diagnosis and treatment regimens of these (Chazya *et al.*, 2014; Kgotlele *et al.*, 2018; Rahman *et al.*, 2018) as well as general small ruminant's management. *Pasteurella multocida* is an opportunistic bacterium causing secondary infection following stress such as cold weather, poor hygienic conditions, concurrent diseases, overstocking and poor housing (Mohamed & Abdelsalam, 2008).

There were few cases involving both SRMV and *M. capripneumoniae* due to the fact that sheep are not natural hosts of *M. capripneumoniae* (Bölske *et al.*, 1995). *Pasteurella multocida* and *M. haemolytica* in some cases concurrently infected both goats and sheep as secondary infecting bacteria, following either CCPP or PPR outbreaks. However, in some cases these were only detected following stressful conditions.

Nasal discharges, diarrhea, anorexia, difficult breathing, reluctance to move and coughing were clinical signs observed in many concurrent infections in goats as previously reported (Taunde *et al.*, 2019; Teshome *et al.*, 2019). Concurrent infections which involved detection of *M. capripneumoniae* were more associated with difficult breathing, reluctance to move and emaciation. On the other hand, asymmetric lung inflammation was the distinguishing postmortem feature that involved *M. capripneumoniae* and opportunistic bacteria concurrent infections. Small ruminants morbillivirus was more associated with anorexia and diarrhea. Vesicular lesions on the gums were associated with most of concurrent infections which involved SRMV with signs linked to involvement of respiratory and digestive systems, respectively (Hamdy *et al.*, 1976).

On the other hand, there were cases where only *P. multocida* and *M. haemolytica* concurrently affected goats and sheep, these are the cases where possibly stress conditions were involved (Mohamed & Abdelsalam, 2008; Rawat *et al.*, 2019).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

A number of risk factors were associated with occurrence of *M. capripneumoniae* and SRMV in small ruminants. However, mixing of species in goats was an important risk factor for *M. capripneumoniae* seropositivity whereas, mixing of flocks was an important risk factor for occurrence SRMV in both goats and sheep. The current diseases surveillance reports does not provide reliable accurate data as supported by the results in this work. However, evidence of improved surveillance reports was observed when field diagnosis is supported with a checklist of possible clinical signs and postmortem lesions of possible diseases. Concurrent infections have been detected in most outbreaks of diseases presenting with respiratory signs and *Pasteurella multocida* being involved in most of the outbreaks in both goats and sheep.

5.2 Recommendations

This study has established that the risk factors associated with *M. capripneumoniae* and SRMV occurrence are day to day practices, therefore recommends that sensitizing on vaccinations will help in controlling the diseases and due to the intamiggling between goats and sheep, further studies on the role of sheep in the epidemiology of *M. capripneumoniae* infection should be conducted. Since the improved field surveillance forms showed better performance, increase communication between service providers during diagnosis and continued education will improve the quality of reports and hence timely interventions and proper control strategies. Presence of concurrent infections, change of disease dynamics and persistence in outbreak investigations of diseases with respiratory signs, I recommend consideration in the control strategy of the multiple pathogens especially the normal commensals of the respiratory system. The major limitation in this study was a study design. In future studies the microbiome analysis of the respiratory tract pathogens should be considered.

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APPENDICES

Appendix 1: Questionnaire for exploring the risk factors associated with diseases presenting with respiratory signs

Background information

Diseases presenting with respiratory signs are the major diseases affecting small ruminants causing significant losses due to high morbidities and mortalities and reduced productivity due to impaired growth, low milk and meat production. In other areas, diseases presenting with respiratory signs can cause mortalities of up to 100% in naive population and up to 20% in endemic areas. Control and diagnosis of these diseases face a great challenge and the high mortalities leave farmers helpless and many of them loose hope because of the losses they incur. This study aims at determining the risk factors for occurrence of selected diseases presenting with respiratory signs, magnitude of diagnostic accuracy of the field surveillance report, farmers' knowledge and perception and the prevalence of the diseases.

Questionnaire

1.0. Household information

1.1 Name of the Head of Household…….

1.2 Sex i) Male ii) Female (……)

1.3 Age (……..…).

1.4 Marital status i) Single ii) Married iii) Widower iv) Widowed (……)

1.5 Interviewee's names……. (If different from 1.1 above)

1.6 Sex i) Male ii) Female (……)

1.7 Age (……..…)

1.8 Educational level i) Primary School ii) Secondary School
iii) Tertiary Institution iv) Others specify (……)

1.9 Name of Village……

1.10 Ward name ……

1.11 Division……Ten Cell Leader……..

2.0. Geographic information

2.1. GPS coordinates **S** ……. **E** ……Altitude (m)……

2.2. Distance to nearest neighbor (approximate km)

i) 2 km ii) 2-5 km iii) 5-10 km iv) more than 10 km (……)

2.3. Distance from village centre (approximate km)

i) ≤2 km ii) 2-5 km iii) 5-10 km iv) more than 10 km (……)

3.0. Herd/flock management

3.1.What type and number of animals do you keep?

Animal type	**Animal Numbers**
i. Goats	(……..)
ii. Sheep	(……..)
iii. Cattle	(……..)
iv. Others (specify)	(……..)

3.2. How do you increase the flock size?

i) Within flock stock reproduction……..

ii) Purchase……

iii) Receive as a gift from friends……. (……)

iv) Receive as dowry…….

3.3. How many animals gave birth in the past 12 months?

No. of animals/period	**Wet**	**Dry**	**Not sure**
i. Goats	(……..)	(……..)	(……..)
ii. Sheep	(……..)	(……..)	(……..)
iii. Cattle	(……..)	(……..)	(……..)

3.4. Where do your animals give birth?

i) Around homestead…… (……)

ii) In pasture lands……

3.5. How many newborns survived after birth in the past 12 months?

No. of animals/period	**Wet**	**Dry**	**Not sure**
i. Goats	(……..)	(……..)	(……..)
ii. Sheep	(……..)	(……..)	(……..)
iii. Cattle	(……..)	(……..)	(……..)

3.6. What happened to those who did not survive?

i) Got sick and died……

ii) Were killed by wild animals……. (…….)

iii) Were lost

iv) Others (specify)……..

3.7. Do you sell animals in the auction markets?

i) Yes……..

ii) No…….. (……)

3.8. What happens to the animals that do not attract buyers?

i) Take them back home…….

ii) Give to friends…….. (……..)

iii) Slaughter and consume with friends at the market……..

iv) Slaughter and sell the meat……..

3.9. Do you buy healthy animals from the auction animals?

i) Yes…….

ii) No……. (……) if Yes go to 3.10, if No go to 3.11

3.10) When did you buy animals for the last time?

i) Within the last twelve months…….

ii) Within the last six months…….

iii) Within the last three months……. (……)

iv) Within the last two months…….

v) Within the last month…….

3.11) Is there any reason for not buying healthy animals

i) Have enough animals…….

ii) Do not want to bring diseases home…….

iii) Do not want to use my money……

iv) Others (specify)……

3.12) Have you ever seen animals dying as a result of difficulties in breathing?

i) Yes…….. ii) No…….. (……) if Yes go to 3.13.

3.13) What diseases could be associated?

i)…….

ii)……

iii)……

iv)……

4.0. Knowledge, attitude, perception and beliefs

4.1. Have you ever seen animals with respiratory diseases? i) Yes ii) /No) (……..). If No go to 4.3

4.2. If **Yes**, mention animals affected by the diseases?

i) .....…….……

ii) …….

iii) ……

4.3. What signs did you see that suggest the animal has a respiratory disease? (**(√ tick**)

Clinical signs	**Yes (V/P)**	**No**
i. Anorexia		
ii. Coughing		
iii. Difficult breathing		
iv. Fever		
v. Reluctance to move		
Serous nasal discharge		
vi. Mucopurulent nasal discharge (could mucoid or even serous discharge)		

V=Volunteered, P= Prompted.

4.4. Do you know the diseases that affect the respiratory systems? i) Yes ii)/No) (……..).

4.5. Can you name them?

i)……..

ii)…….

iii)……

iv)……

v)…….

4.6 Can these diseases be prevented/controlled from affecting animals?

i) Yes…….. ii) No …… If Yes, go to 4.7

4.7 How can these diseases (in 4.5) be prevented/controlled? **(√tick where** appropriate)

Control measure	i	ii	iii	iv	v
i. Vaccination					
ii. Avoid introducing animals from other herds and mixing at the communal grazing land					
iii. Prevent animals from drinking contaminated water / sharing of the communal drinking areas					
iv. Treat them with antibiotics					
v. Slaughter all weak animals					
vi. Prevent animals from drinking water in the communal pond					
vii. None					
Other (specify)…….					

4.8 What are activities associated with occurrence of diseases mentioned in 4.5?

Activity	**i**	**ii**	**iii**	**iv**	**v**
Purchase of animals from auctions/markets					
Grazing in the communal land					
Drinking water in the communal ponds					
Contact with other new animals					
Trekking for long distance					
Transportation of animals for long distance					
Others (specify)					

5.0. Livestock movement and contact structure

5.1 How frequently your animals mix with other flocks?

i) Often……..

ii) Occasionally…….. (……..).

iii) Never…….

HERDS	DRY SEASON		WET SEASON	
	Grazing areas	**Watering points**	**Grazing areas**	**Watering points**
Goats				
Sheep				
Cattle				
Wild animals				
Others (Specify				

5.2 Are your sheep and goats flocks mixed with other sheep and goats from other bomas?

i) **Yes**…….. ii) No……. (……)

5.3 Did you bring in (purchase, dowry or gift) goats and sheep in 2015/16?
(i)Yes…… ii) No……. (……..).

5.4 If **Yes;** indicate the origin and numbers acquired

	Village	**Total**	**Market**	**Total**	**District**	**Total**	**Others (specify).**	**Total**
Goats								
Sheep								

6.0 Family activities

6.1. Who is responsible for the following activities?

	Assisting newborns (Yes/No/NA)		**Herding** (Yes/No/NA)		**Purchasing** (Yes/No/NA)		**Milking**		**Selling**	
	Goats	Sheep	Goats	sheep	oats	sheep	Goats	sheep	Goats	sheep
Husband										
Wife										
Son										
Daughter										
Attendant (employee)										
Others (specify)										

7.0 Specific animals – suspected cases

7.1. Animal information

ID No/name	Age	Type	Colour	Species	Breed	Weight

7.2. Animals' reproductive history and newborn status

	Animals ID No/name (from 7.1)	Kidding /Lambing Record	Kids/Lambs alive	Kids/Lambs dead	Reason of deaths
Goats					
Sheep					

7.3. Treatment records

7.3.1. Can you pick a sick animal(s) from the flock? (Yes / No)……

7.3.2. Mention the reasons that will guide you in picking the sick animal from the flock

i) .....
.

ii) .....
.

iii) .....
.

iv) ……

7.3.3. For how long have you seen the reasons you mentioned in 7.3.2……

7.3.4. Were the sick animals identified in the flock treated? (Yes / No)……

7.3.5. Who treated the animal? (Myself/Veterinarian)……

7.3.6. When the animal is sick and you request assistance from a professional, how long does it take for him her to come?

i) Within a day

ii) After two days

iii) After a week

iv) Others (specify)

7.3.7. If the professional delays or he/she does not come who treats the sick animal?

i) Husband…….

ii) Wife……

iii) Neighbour……..

7.3.8. After the course of treatment of the sick animals, what were the results of treatment?

i) Died

ii) Recovered

iii) slaughtered

7.3.9. Do you have any comments or questions regarding the control of diseases presenting with respiratory signs in small ruminants?

Appendix 2: Animal disease field surveillance form [FD-1]

THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF LIVESTOCK AND FISHERIES DEVELOPMENT

VETERINARY SERVICES

ANIMAL DISEASES SURVEILLANCE FIELD REPORT

ZONE		REGION	
DISTRICT		WORK STATION	
VILLAGE			

GRID REFERENCE

LATITUDE	
LONGITUDE	
ELEVATION	

DISEASE NAME

FOLLOW UP REPORT

CONTAGIOUS BOVINE PLEUROPNEUMONIA		YES		NO	

DATE	SPECIES AFFECTED	EPIDEMIOLOGY			DISEASE CONTROL		
		NUMBER OF ANIMALS					
		At risk (total in the village)	Cases	Deaths	Vaccinated	Treated	Destroyed

DIAGNOSIS		AFFECTED POPULATION				PRODUCTION SYSTEM	
		SEX		AGE		Agro-pastoral	
Suspected		All		<6 Months		Com.beef	
Clinical		Female		6M -1yr		Com.Dairy	√
Laboratory		Male		Adults		Zero/intensive	√
Post-mortem		Castrate		All			
Meat inspection							

Other control measures	Main clinical and PM features

Epidemiology of the disease:

Position

VET		LFO	√
LO			
AHA			

Signature…… Full Name …….

Appendix 3: Animal diseases abattoir report form

UNITED REPUBLIC OF TANZANIA

MINISTRY OF LIVESTOCK DEVELOPMENT AND FISHERIES

LIVESTOCK DEPARTMENT

VETERINARY SERVICES

ABATTOIR REPORT

ZONE

REGION DISTRICT

VILLAGE

Date of Inspection

LAT

GRID REF

LONG

NAME OF ABATTOIR

Owner of Animals & Address

Origin of Animals

Region

District

Village

Species of Animals

No. of Animals Affected | | Slaughtered No. of Animals | |

Age Affected

All	
Adult	
6 Months – 2 years	

Sex Most Affected (Please tick)

All	
Female	
Male	
Castrates	

Signs observed before slaughter:

Signs and Lesions observed after slaughter:

Disease suspected/Diagnosed | |

Signature……..……..

Full Name…….……

Position

Vet Dr.		Meat Inspector
LFO		

Appendix 4: Modified field surveillance form [FD-2]

MODIFIED FIELD SURVEILLANCE FORM

District.....Village.....Date.....

Outbreak No……..... Age……...

Sex…….....

1. **CLINICAL INFORMATION**

1.1. When did the animal first get sick?.....

1.2. Does the animal have clinical signs suggestive of respiratory or digestive systems involvement? Y / N

1.3. If Y, select the clinical sign and describe the severity:

i. Anorexia: Y/N

ii. Vesicular lesions on gums): Y/N

iii. Diarrhoea: Y/N

iv. Coughing: Y/N

v. Nasal discharge: Y/N

vi. Eye discharge: Y/N

vii. Difficult breathing: Y/N

viii. Reluctance to move: Y/N

ix. Emaciation: Y/N

x. Other sign:

xi. Describe…….

……

……

For affected sheep and goats, take photographs of lesions (make sure that the animal can be identified for each photo).

2. PATHOLOGICAL INFORMATION

2.1. When small ruminants die of diseases presenting with respirator signs, tick the pathological lesions depicted at postmortem

i. Consolidation of lung lobes: Y/N

ii. Frothy exudates in trachea and bronchi: Y/N

iii. Straw coloured pericardial fluid: Y/N

iv. Lesions on the organs along the digestive tract: Y/N

v. Congested lining of nasal cavity: Y/N

vi. Firm to touch lobes of the lung: Y/N

vii. Soft, swollen and haemorrhagic lymph nodes: Y/N

viii. Yellow nodules surrounded by congested lung lobes: Y/N

ix. Numerous attachments to the chest wall: Y/N

x. Other lesion(s): Describe……..

…….……

……..……..…

……

For autopsied sheep and goats, take photographs of lesions (make sure that the cadaver can be identified for each photo, e.g. take photo lesions like the zebra stripes, vesicular lesions, consolidated lobes, attachment on the wall, straw coloured fluids). Photo Y/N

3. SURVIVORS CLINICAL INFORMATION

3.1. If the animal is still alive – is the animal still showing any clinical signs of disease? Y/N

3.2. If Yes, list the signs

i. …….....

ii. ……

iii. .....

3.3. And, how many months the clinical signs have persisted?

i. Anorexia: .....

ii. Vesicular lesions on gums): .....

iii. Diarrhoea: .....

iv. Coughing: .....

v. Nasal discharge: .....

vi. Eye discharge: .....

vii. Difficult breathing: .....

viii. Reluctance to move: .....

ix. Emaciation: .....

4. TENTATIVE DIAGNOSIS

4.1. From the clinical signs and/or post-mortem lesions seen what could possibly be the disease(s) involved in the outbreak?

i. .....

ii. .....

iii. .....

iv. .....

v. .....

SAMPLE CHECKLIST – for animals sampled during the outbreaks (you could also add another series of check boxes for samples collected from any animals that died)

Sera in plain vacutainers	☐	EDTA blood at RT.....	☐
Nasal swab.....	☐	Eye swab	☐
Infected lung section	☐	Infected intestine sections)	☐
Tracheal swab	☐		

RESEARCH OUTPUTS

(a) Manuscript(s)/Journal Paper(s)

Chota, A., Shirima, G., & Kusiluka, L. (2020). Diagnostic challenges and control strategies for diseases presenting with respiratory signs in small ruminants in developing countries: Emphasis on Contagious Caprine Pleuropneumonia and Peste Des Petits Ruminants. *International Journal of Tropical Disease and Health, 41*(22), 12-25. DOI: 10.9734/IJTDH/2020/v41i2230409.

Chota, A., Shirima, G., & Kusiluka, L. (2019). Risk factors associated with *Mycoplasma capricolum* subspecies *capripneumoniae* and morbillivirus infection in small ruminants in Tanzania. *Tropical Animal Health and Production*, 51, 1807–1815. https://doi.org/10.1007/s11250-019-01981-4

Chota, A., Shirima, G., & Kusiluka, L. (2020). Detection of contagious caprine pleuropneumonia and concurrent infections in outbreaks of diseases presenting with respiratory signs in small ruminants in Tanzania. *International Journal of Tropical Disease and Health, 41*(7), 70-83. DOI: 10.9734/IJTDH/2020/v41i730302

(b) Conference Papers(s)

Chota, A., Shirima, G., & Kusiluka, L. (2018). *Limited Biosecurity Leaves Vaccination as the Most Feasible Option for Diseases Control in Africa*. Paper presented at the 30th World Congress on Vaccine and Immunization, held in Osaka Japan 22 -23, October, 2018 https://vaccinescongress.vaccineconferences.com/2018/