

The Epidemiology of Foot-and-Mouth Disease in the Kingdom of Bhutan

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This thesis is presented for the degree of Doctor of Philosophy of Murdoch University

2011

Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Abstract

Foot-and-mouth disease (FMD) is a highly infectious viral disease of all cloven-hoofed animals. It can have a significant impact on the livelihood of livestock owners, especially in developing nations such as Bhutan.

Prior to the study reported in this thesis, there was limited understanding of the epidemiology of FMD in Bhutan in terms of its spatiotemporal distribution, risk factors, role of animal movement, and disease surveillance. Retrospective and prospective studies were conducted to unravel the epidemiology of FMD in Bhutan in order to support and refine the current control programme.

The study demonstrated that FMD is endemic and periodically epidemic in Bhutan with the districts and sub-districts bordering India being at higher risk of disease than the interior districts. The districts and sub-districts bordering India appear to behave like primary endemic areas for the introduction and persistence of FMD virus through frequent unofficial movements of cattle across the porous border. The interior districts and sub-districts appear to behave like secondary endemic areas where virus propagation occurs due to limited vaccination coverage and unrestricted movement of animals.

The study showed that O was the principal serotype in Bhutan, consistent with the disease epidemiology in the neighbouring countries. Cattle are the most susceptible animal while small ruminants and pigs seem to have minor roles in the disease's epidemiology. However, unvaccinated small ruminants can be used as tracers for disease surveillance in areas where cattle are routinely vaccinated. Waves of outbreaks of FMD, in cyclical patterns, have occurred in Bhutan due to the incursion of the PanAsia strain of the O serotype, possibly through transboundary movement of livestock from neighbouring countries. The devastating capacity of the PanAsia strain of the O serotype, especially in a

FMD-naïve population, was shown through the large scale morbidity and mortality of cattle and pigs during the 2007 epidemic in Bhutan. The disease produced significantly higher morbidities and mortalities in Zhemgang district (36.5% vaccination coverage) as compared with Sarpang district (87.6% vaccination coverage).

Husbandry practices, such as mixing of cattle within and between villages at grazing and watering areas, and feeding kitchen wastes to cattle significantly increased the risk of transmission of FMD in FMD-endemic herds. The seroprevalence of FMD in the migratory herds (24.8%, 95% CI: 20.6, 29.5) was significantly higher than in the sedentary herds (17.5%, 95% CI: 15.6, 19.5) thus underlining the significance of this livestock production system for the disease's epidemiology.

Animal movements occurred in several forms including the daily movement of animals within and between villages for grazing and watering purposes; livestock trading within and between villages, sub-districts, and districts; and the traditional migratory practices. All these movements pose significant risks for disease transmission given the ineffective regulatory and quarantine services. The animal movement patterns were more complex in an FMD-endemic district (Sarpang) compared with an FMD-free district (Tsirang). There were more inward than outward movements for all species in the endemic district as compared with the FMD-free district. The presence of numerous unofficial trading routes along the Indo-Bhutan border in Sarpang district could be an important determinant for the frequent incursion and persistence of FMD in this district.

Active serological and questionnaire-based surveys have validated the usefulness of the country's passive surveillance system. Although the current findings have increased the level of confidence in the passive surveillance system of Bhutan, there is a need to complement this with active serological and clinical surveys from time to time.

Several factors, such as extensive livestock husbandry practices, rugged terrain, inadequate vaccination coverage, ineffective regulation of movement control, porous borders, a lack of awareness of the disease by the farming community, and budgetary constraints, pose significant challenges to the prevention and control of FMD in Bhutan. Given the disease's endemicity, controlling FMD for the whole country is currently both difficult and costly. Therefore alternative approaches using the concepts of zoning are proposed. Longitudinal studies, using active serological and clinical surveillance, indicated the absence of FMD infection in the district of Tsirang at the time of this study. This has now paved the way for initiation of progressive zoning approaches as an alternative control method in line with the global framework for the control of transboundary diseases.

It is concluded that a regional approach is needed in order to successfully control this transboundary disease in Bhutan and neighbouring countries.

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Acknowledgements

This thesis would not have been completed had it not been for the support of so many individuals and institutions.

Firstly, I wish to thank Professor Ian Robertson, my principal supervisor, for his constant guidance, academic critiquing, encouragement, and timely feedback. I also thank him for allowing me to sit in his Epidemiology classes that enabled me to learn new skills and tools. My sincere appreciation to Dr. Trevor Ellis, co-supervisor, for his words of wisdom and constant feedback at all stages of my project. I owe my gratitude to Professor John Edwards for facilitating my enrolment at Murdoch and providing me this wonderful opportunity of furthering my studies in Australia. I thank the Australian Government (Australian Biosecurity Cooperative Research Centre and Murdoch University) for the generous financial support.

I would like to thank the Director General of Livestock Department, Dasho Tenzin Dhendup, for supporting my study. I appreciate the support of my colleagues: Drs Pasang Tshering, Karma Rinzin, Narapati Dahal, Vijay Raika, Nirmal Thapa, Phuntsho Wangdi, Rinzin Pem, Basant Sharma, S B Chamling, Tenzin, and Birdoj Rai for facilitating my field and laboratory works in Bhutan. I thank the District Livestock Officers, field extension officers, laboratory technicians, and other support staff who were involved in my project. Thanks to Dawala and Purna Rai for undertaking the NSP and the LPBE tests.

My sincere appreciation to Chris Morrissy, Ross Lunt, and Leanne McNabb, of the Australian Animal Health Laboratory, for organising the NSP and LPBE tests. Thanks to Drs. Shih-Ping Chen and Michael Lee, of the Animal Technology Institute, Taiwan, for

providing training on NSP tests and free supply of NSP kits. Thanks to Dr. Yeou-Liang Lin of the Animal Health Research Institute, Taiwan for organising training on the LPBE.

I am indebted to Dr. Evan Sergeant, Epidemiologist, AusVet Animal Health Services, Australia, for his expert opinion and advice at all stages of my project.

My three-and-half years of stay in Perth wouldn't have been so smooth without the support of my Bhutanese and Australian friends. Thanks to Aue Tshering and Aum Ugyen, Kinzang Tobgay and Aum Sangay, Nima and Aum Tshering (“Chini”), Thinley (“Khotsa”), Tenzin and Aum Karma, Karma Lhagyel, and Mindu, for their unflinching support to my family, particularly during my field work in Bhutan. Thanks to Tsheten for helping me to unwind in the midst of my busy writing sessions. Thanks to Sherab Namgay and Aum Tashi, Wangdi and Aum Tashi Pedon, Kado and Rinzin, Hotem, Patsy and Glen, Tashi Tenzin and Aum Moenlam, for their friendly company. Thanks to the “trailer-trash” peers: Kyaw (Myanmar), Tum Sothyra (Cambodia), Siti (Malaysia), Jim (Philippines), Acacio (East Timor), Jarunee (Thailand), and Peter Adams for their comradeship.

I am thankful to my late father (Gopa Sangay), mom (Dawa Bidha), aunt (Gyem Lham), brothers (Thinley and Ugay), other relatives and family friends for their encouragement and for bearing with our absence for this long.

I appreciate the support and understanding of my beloved wife, Tshering, and my two gorgeous children, Tashi Kinzang Wangchuk and Dechen Kinzang Lham, for their unrelenting love, patience, and encouragement throughout this project. I acknowledge the selfless effort made by Tshering in taking excellent care of our children while I was engrossed in my work.

This thesis is dedicated to Tashi and Dechen.

TASHI DELEK

Acronyms and Abbreviations

AAHL	Australian Animal Health Laboratory
ANOVA	Analysis of variance
BAFRA	Bhutan Agriculture and Food Regulatory Authority
c-ELISA	Competition ELISA
CI	Confidence interval
DLO	District Livestock Officer
DOL	Department of Livestock
<i>Dzongkhags</i>	Local name for Districts in Bhutan
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organisation
FMD	Foot-and-mouth Disease
FMDV	Foot-and-mouth Disease Virus
<i>Geogs</i>	Local name for Sub-districts in Bhutan
<i>Gup</i>	Head of a sub-district in Bhutan
LABSYS	Laboratory Systems database of Bhutan
LEC	Livestock Extension Centre
LPBE	Liquid phase blocking ELISA
<i>Mangmi</i>	Deputy head of a sub-district in Bhutan
m.a.s.l.	Metres above mean sea level
MEP	Minimum expected prevalence
NCAH	National Centre for Animal Health
NFMDCP	National FMD Control Programme of Bhutan

NSP	Non-structural protein
OIE	World Organisation for Animal Health (Office International des Epizooties)
OR	Odds ratio
PI	Percent inhibition
RLDC	Regional Livestock Development Centre
RNREC	Renewable Natural Resources Extension Centre
RT-PCR	Reverse transcriptase polymerase chain reaction
SD	Standard deviation
SEACFMD	South-East Asia and China FMD Control and Eradication Campaign
SEM	Standard error of the mean
SP	Structural protein
SPCE	Solid phase competition ELISA
SPSS	Statistical programme for social sciences
SVL	Satellite Veterinary Laboratory
TAD	Transboundary animal diseases
<i>Tshogpa</i>	Head of a village in Bhutan
VNT	Virus neutralisation test
VIS	Veterinary Information System of Bhutan
WRLFMD	World Reference Laboratory for FMD

CHAPTER ONE

Introduction

1.1 Introduction

The livestock sector constitutes an important component of agriculture for poverty alleviation as a large proportion of the poor keep livestock as a source of food, income, manure, draught power, social status, a buffer against risk and a form of savings (FAO, 2008). Livestock contributes significantly to the world economy as approximately 40% of the global agricultural gross domestic product comes from livestock and approximately 1.3 billion people are employed in the industry (Steinfeld *et al.*, 2006). Increased livestock productivity results in a positive impact on the lives of those in developing countries (Forman *et al.*, 2009). However, animal diseases have been identified as one of the major constraints to increasing livestock productivity. Highly contagious transboundary diseases, such as foot-and-mouth disease (FMD), can have severe economic and social effects (James and Rushton, 2002).

Foot-and-mouth disease is a highly contagious viral disease which affects all cloven-hoofed domestic animals including cattle, sheep, goats, pigs and buffalo. The disease is endemic in many parts of the world, particularly in developing countries of Asia, Africa, the Middle East, and some parts of Europe. Foot-and-mouth disease is endemic in Bhutan (Sharma, 1992) and causes economic hardship to the livestock farmers.

In FMD-free countries that are dependent on livestock trade, an outbreak of FMD can have severe economic consequences in terms of trade restrictions and costs of disease control

and eradication. For instance, Taiwan suffered a loss of approximately four billion US dollars, 90% of which was from lost export earnings, due to the widespread outbreaks in 1997 (Kitching *et al.*, 2007). The cost of the 2001 outbreak to the UK economy was over eight billion US dollars (Thompson *et al.*, 2002). In FMD-endemic countries that are not dependant on earnings from the export of animals and animal products, the economic consequences of an outbreak of FMD may not be obvious. In Bhutan, where 90% of the farmers depend on livestock-related farming for their sustenance (Anonymous, 2007a), the disease can have a direct effect on the farmers' livelihood in terms of reduction in production (milk and milk products) and loss of draught power.

The control strategies adopted in countries free from FMD include "stamping-out" or culling of infected animals, selective vaccination, disease surveillance and movement restriction of animals and animal products.

In Bhutan, vaccination (prophylactic and post-outbreak) has been the main strategy adopted for the prevention and control of FMD. Other control strategies include regulation of the movement of livestock and livestock products during outbreaks; clinical surveillance; application of zoo-sanitary measures; and awareness programmes for the farming community.

Bhutan continues to experience outbreaks of FMD almost every year, despite concerted effort by the government to control the disease. Several factors have been cited as reasons for this failure including inadequate vaccination coverage, ineffective vaccination due to difficulties in maintaining cold chain of vaccines, difficulties in the enforcement of bans on livestock movements during outbreaks, and a lack of knowledge on the epidemiology of the disease (Tshering, 2003, Anonymous, 2005). The success of any disease control programme depends on a thorough understanding of the disease's epidemiology. Studies on local epidemiological characteristics of FMD can provide valuable insights into

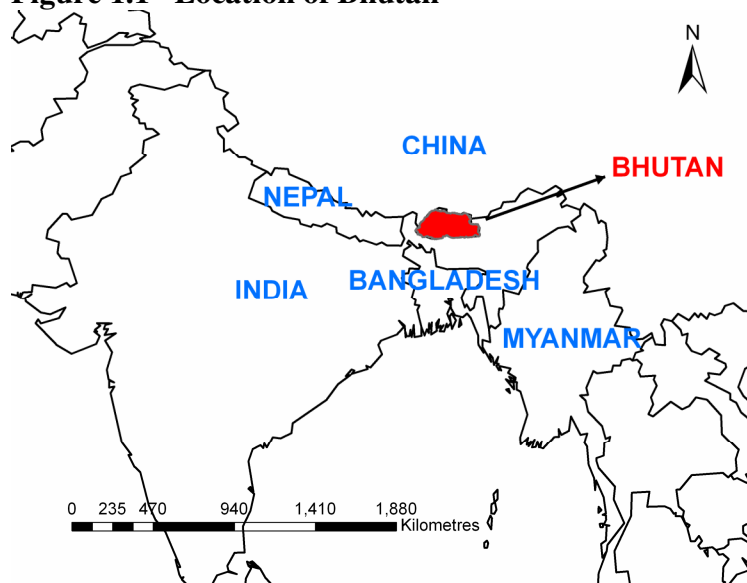
different aspects of the disease patterns and can be useful for future disease control programmes (Gibbens *et al.*, 2001, Moutou, 2002, Gallego *et al.*, 2007). A good understanding of the disease's epidemiology is essential for the development of efficient FMD surveillance, control and eradication programmes (Al Khamis *et al.*, 2009).

1.2 Background information on Bhutan

1.2.1 Geo-physical and socio-economic facts

The Kingdom of Bhutan is a small landlocked country nestled in the Himalayas and sandwiched between China to the north and India to the south (Figure 1.1). With an area of 38,394 square kilometres, the distance between north-south and east-west stretches approximately 170 and 300 kilometres, respectively (Anonymous, 2007d). In 2008, Bhutan had a human population of 671, 083 (Anonymous, 2009c) of which 79% lived in villages depending on agriculture for their livelihood (<http://www.bhutan.gov.bt/government/aboutbhutan.php>, accessed on 10th September 2009). Bhutan, with a population density of approximately 16 people per square kilometre (<http://www.bhutan.gov.bt/government/aboutbhutan.php>, accessed on 10th September), is one of the least densely populated countries in South Asia.

Figure 1.1 Location of Bhutan



Administratively, the country is divided into 20 districts (*Dzongkhags*); each district being further sub-divided into sub-districts (*Geogs*) of which there were 205 in 2010. Each sub-district contains several villages. Bhutan is predominantly a mountainous country and has a diverse topography with altitudes ranging from as low as 160 metres above sea level (m.a.s.l.) in the south to as high as 7500 m.a.s.l. in the north.

1.2.2 Agro-climatic zones of Bhutan

Due to extreme altitude variations over short distances, the country experiences diverse climatic conditions ranging from wet sub-tropical conditions in the south to alpine conditions in the north. The country can be divided into six agro-ecological zones (Table 1.1) based on altitude, temperature and rainfall (Dorji, 1995).

Table 1.1 Agro-ecological zones of Bhutan

Agro-ecological Zones	Altitude (m.a.s.l.)	Temperature (°C)			Average rainfall (mm)
		Maximum	Minimum	Mean	
Alpine	>3500	12.0	-1.0	5.5	<650
Cool temperate	2500-3500	22.0	1.0	10.0	650-850
Warm temperate	1800-2500	26.0	1.0	13.0	650-850
Dry sub-tropical	1200-1800	29.0	3.0	17.0	850-1200
Humid sub-tropical	600-1200	33.0	5.0	20.0	1200-2500
Wet sub-tropical	150-600	35.0	12.0	24.0	2500-5500

In general, four seasons are found in Bhutan: Spring from February to April (cool and dry), summer from May to July (warm and humid), autumn from August to October (cool and humid), and winter from November to January (cold and dry).

1.2.3 Agricultural production systems

Agricultural development has been constrained mainly due to a lack of arable land. Due to rugged terrain and mountainous topography, only 8% of the total country is arable and

only approximately 4% is pasture land (Anonymous, 2007d). The contribution from the agricultural sector to the Gross Domestic Product for 2008 was 18.9% (Anonymous, 2009c), thus indicating the importance of this sector to the country. Livestock farming forms an integral and indispensable part of the agricultural farming system in the country with approximately 90% of the agrarian population owning livestock for various agricultural activities (Anonymous, 2007a).

Agriculture in Bhutan is still largely subsistence orientated, although efforts are being made to intensify agricultural production. The agricultural production system is primarily dictated by the agro-ecological conditions and topographical features (Anonymous, 2002b). A pastoral production system with yak-rearing is the main source of livelihood for the semi-nomadic population in the alpine zone. In the cool temperate areas, livestock still constitute a major source of income for farmers, although crops, such as potatoes, buckwheat, barley and wheat, are also grown (Anonymous, 2002b).

A wide range of crops, including rice, barley and potatoes and fruits such as apples, pears, and peaches, are grown in the warm temperate zone. While livestock in this zone continue to be the main source of manure and draught power, farmers are increasingly adopting farm mechanization and chemical fertilizers. In the dry sub-tropical zone, maize is the most common cereal followed by millet and pulses. The humid and wet sub-tropical zones are very important wetland rice production areas. In all agro-ecological zones, farmyard manure, mainly from cattle, still constitutes the major source of farm nutrients for agricultural production (Anonymous, 2002b).

1.2.4 Livestock production system

Livestock farming is still predominantly subsistence where farmers rear only a few animals, mainly cattle, to support their income through the sale of livestock products.

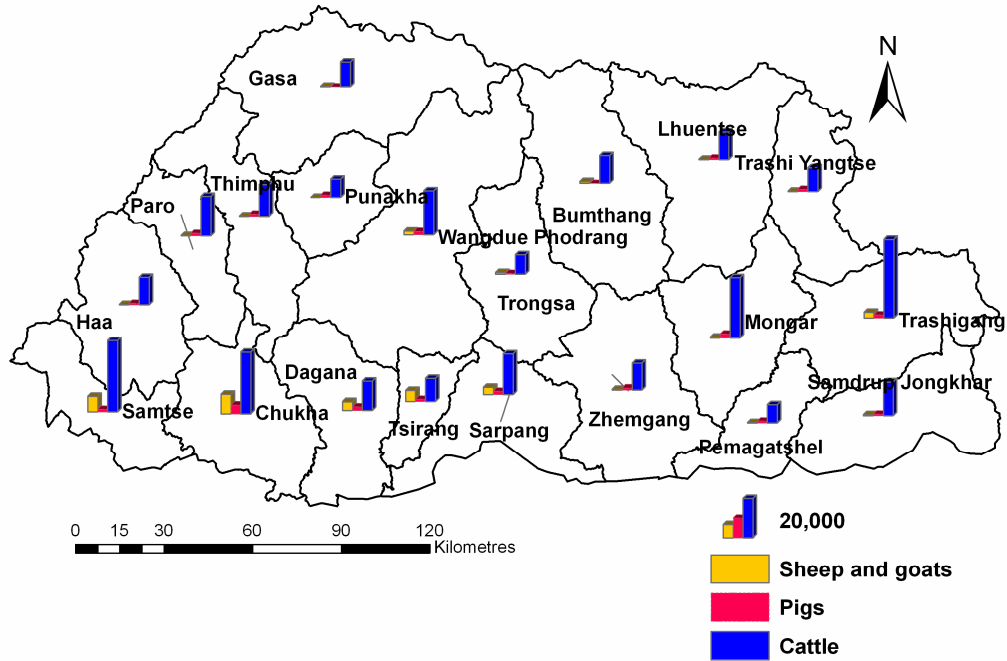
However, increasingly, farmers in the peri-urban areas in the towns and cities have started to intensify livestock farming mainly in dairy and poultry production to meet the growing demand for dairy and poultry products. The type of livestock reared is directly dependent upon the agro-ecological as well as the social conditions. In the dry-subtropical and wet-subtropical zones, where the climate is hot and humid, farmers rear multiple species including cattle, sheep, goats, poultry and pigs. In the temperate zone, farmers rear mostly cattle whereas in the alpine zone, yak-farming is the only livestock activity feasible due to the extreme cold.

1.2.5 Livestock population

In 2007 Bhutan had a total of 440,631 FMD-susceptible domestic animals (Anonymous, 2009a). Cattle (72.6%) were the most common animal followed by yaks (*Poephagus grunniens*, 11.7%), goats (6.4%), pigs (6.1%), sheep (2.8%), and buffalo (*Bubalus bubalis*, 0.4%).

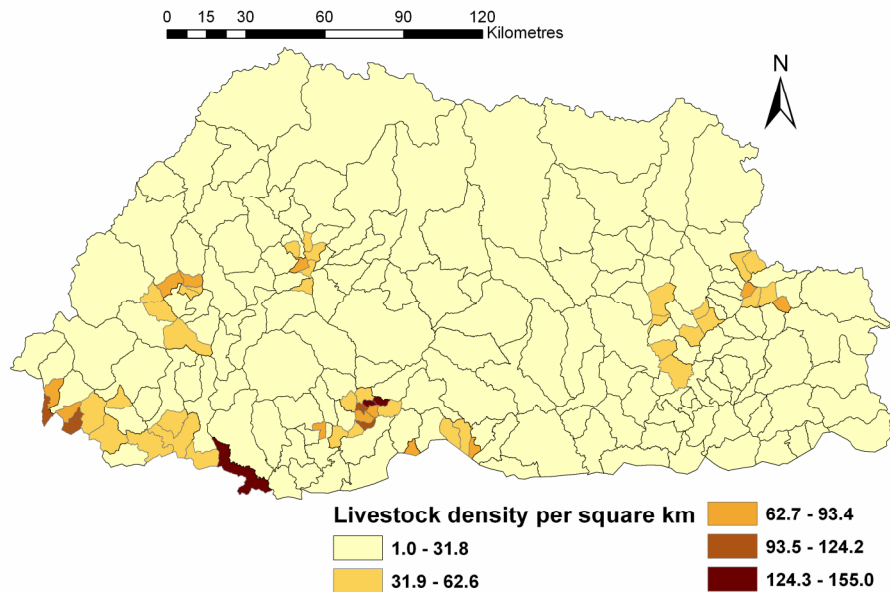
The distribution of FMD-susceptible species (2007 census) in different districts is displayed in Figure 1.2.

Figure 1.2 Distribution of FMD-susceptible species at the district level



The distribution of FMD-susceptible livestock (all species) at the sub-district level per square kilometre is displayed in Figure 1.3.

Figure 1.3 Distribution of FMD-susceptible livestock population at the sub-district level



1.3 Veterinary services in Bhutan

Veterinary services in Bhutan are completely owned by the government. All animal health care activities, such as clinical services, prevention and control of diseases, supply of medicines and vaccines, are provided freely by the government.

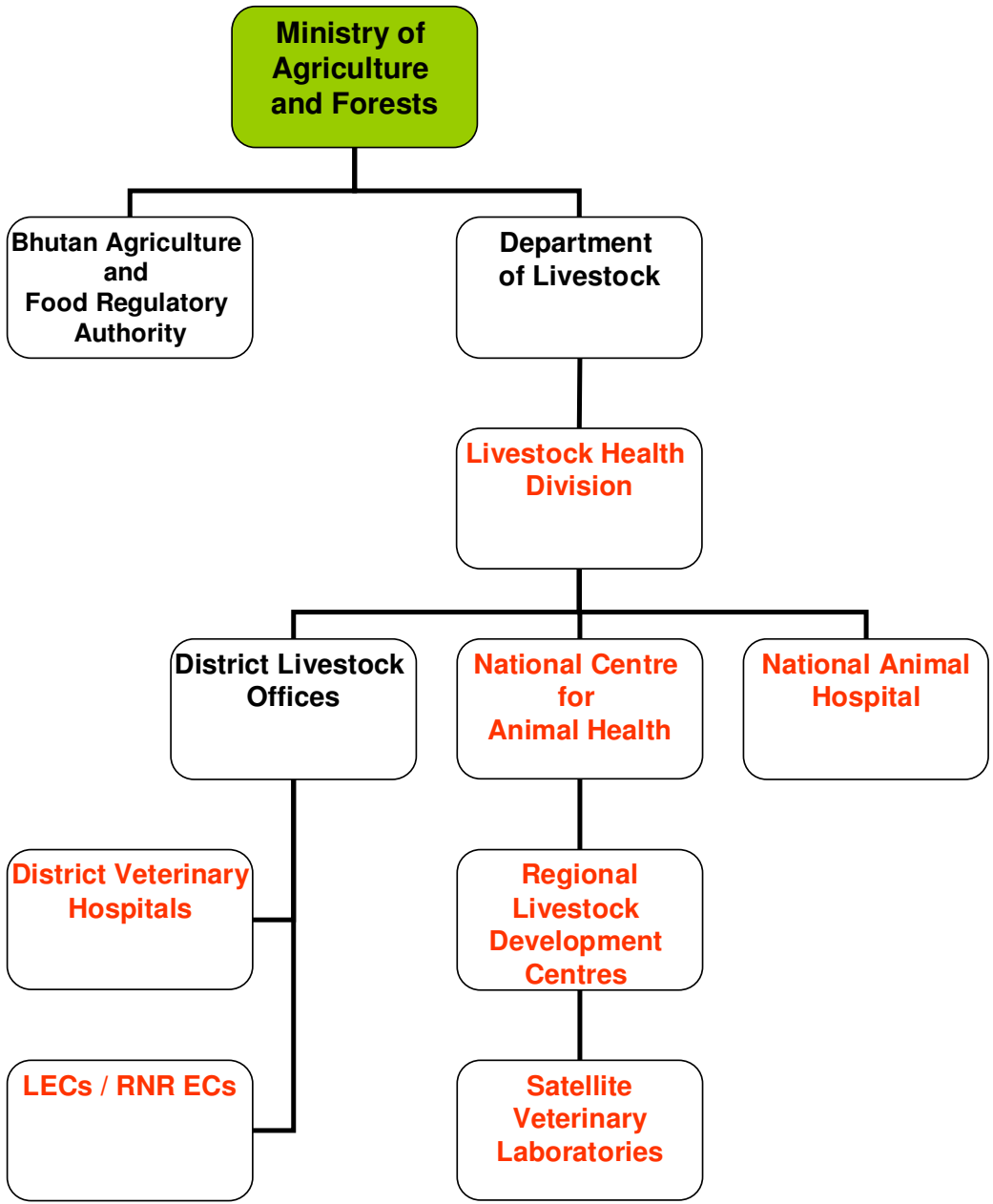
The Department of Livestock (DOL) is the agency responsible for the overall livestock developmental activities in the country. The structure of the veterinary services in Bhutan is depicted in Figure 1.4 and the location of the institutes/organisations responsible for delivery of veterinary services is displayed in Figure 1.5.

The delivery of frontline animal health services, including treatment, vaccination, and surveillance activities, are undertaken by the Livestock Extension Centres (LECs) or Renewable Natural Resources Extension Centres (RNRECs) which are located in all 205 sub-districts. The LECs cater only to animal health and livestock activities. The RNRECs cover the agricultural, forestry, and livestock sectors and are manned by one diploma professional per sector.

The activities of the livestock sector housed in an RNREC are similar to that of the LEC. These LECs/RNRECs are manned by para-veterinarians and are technically supported by three Satellite Veterinary Laboratories (SVLs), four Regional Livestock Development Centres (RLDCs) and the NCAH (Figure 1.4). The laboratories provide a wide range of services, including laboratory diagnostic services; animal health research; disease surveillance; and training.

The Bhutan Agriculture and Food Regulatory Authority (BAFRA) is mandated to undertake the quarantine and inspection of all livestock and livestock products that are imported or exported, including the internal movement of livestock and livestock products.

Figure 1.4 Organisational structure of the veterinary services (animal health) in Bhutan

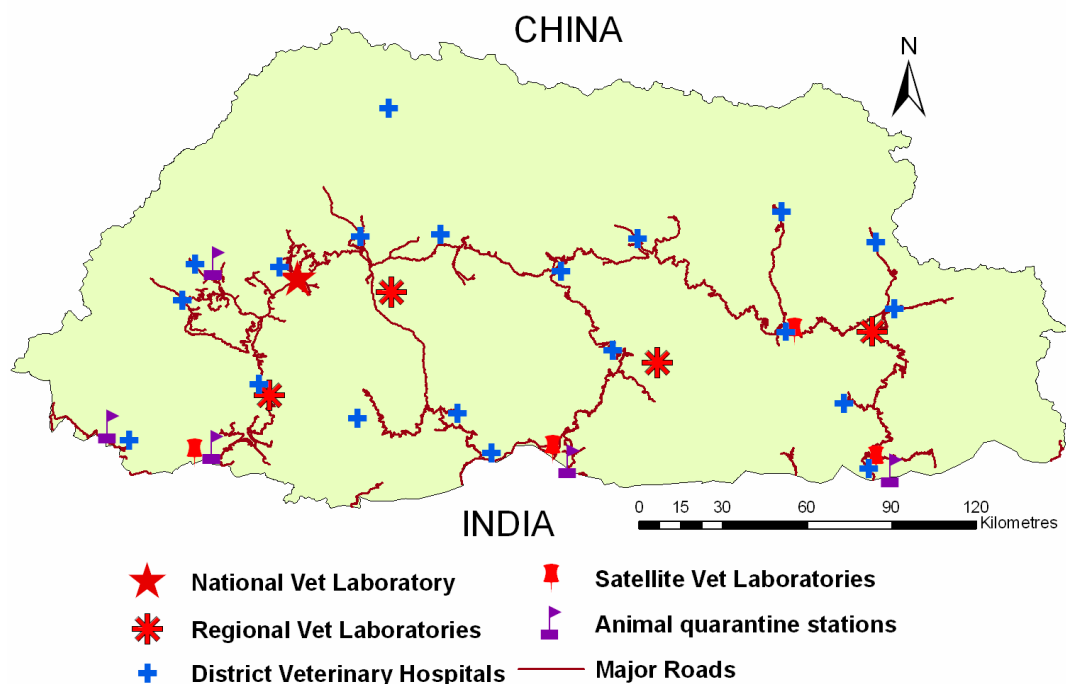


Note: LECs= Livestock Extension Centres; RNRECs = Renewable Natural Resources Extension Centres; Institutes marked in red are those that are directly involved in animal health delivery in Bhutan

The animal quarantine stations located in the four southern border districts and the airport at Paro district (Figure 1.5) are the designated ports for the official quarantining and inspection of live animals imported into the country. Regulatory inspectors from BAFRA are posted in most of the police check points along the major highways. The DOL is

mandated to set the standards for sanitary and phytosanitary requirements for all activities relating to livestock and livestock products, while the BAFRA implements, monitors, and regulates these standards.

Figure 1.5 Location of animal health institutes/veterinary centres in Bhutan



1.3.1 Surveillance system

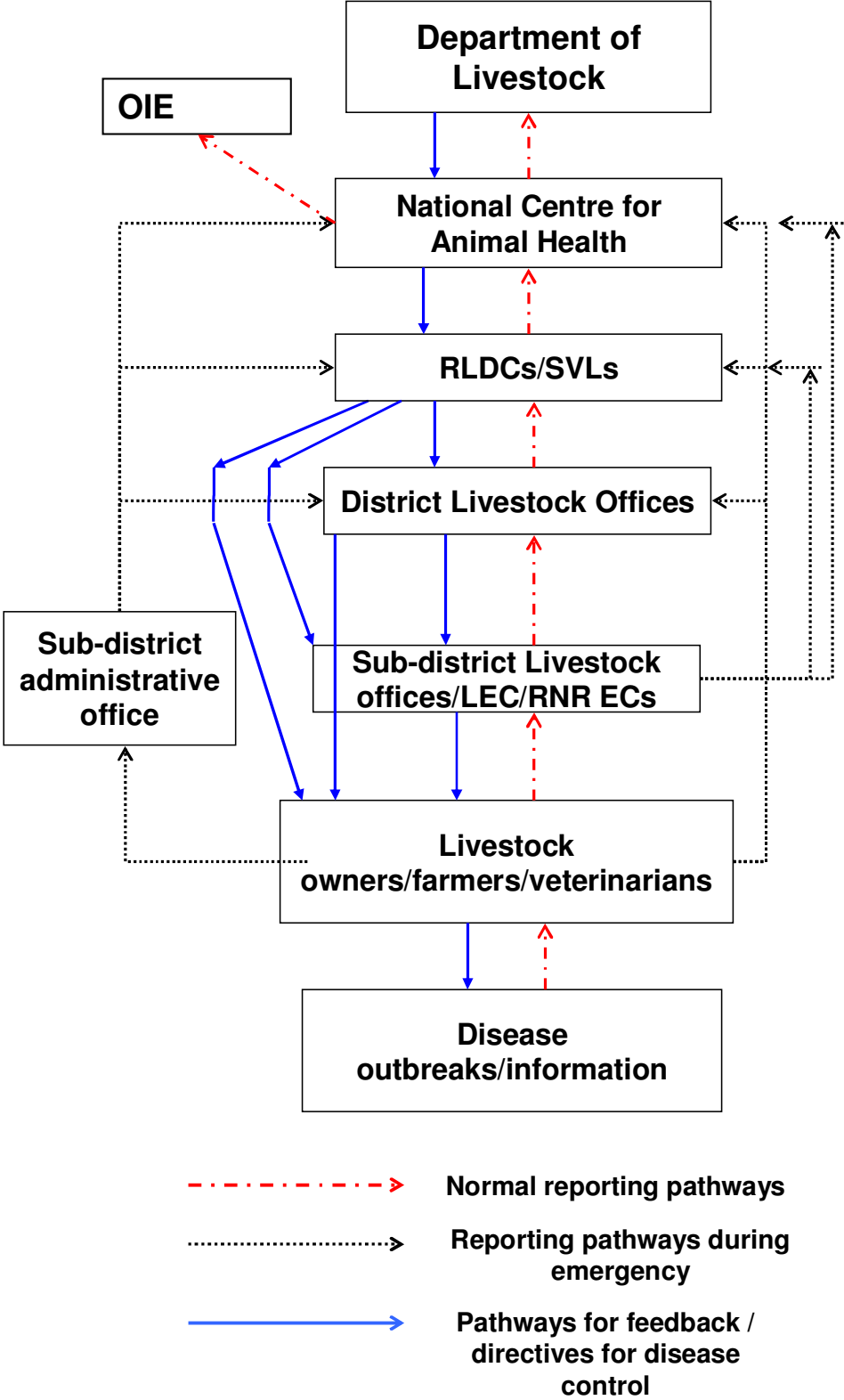
The country’s passive surveillance system is essentially based on farmer-diagnosed and reported presence of diseases in their herds and villages. The flow of information starts from the point when a farmer reports an occurrence of disease (infectious or non-infectious) to the nearest LEC or when a farmer submits laboratory samples (faecal) to the SVLs/RLDCs (Figure 1.6). In the event of the occurrence of infectious diseases, such as FMD, the farmer can directly report to the SVLs/RLDCs/NCAH, or through the heads of sub-districts (*Gups*) or LECs/RNRECs. By law, it is mandatory for the administration at the village and sub-district level to report cases of notifiable diseases such as FMD, Black

Quarter (BQ), Haemorrhagic Septicaemia (HS), Newcastle disease (NCD), Avian Influenza (AI), Swine Fever (SF), and Anthrax to the nearest LECs/SVLs/RLDCs.

Following an outbreak of infectious disease, such as FMD, the LECs/RNRECs are required to inform the SVLs/RLDCs of the outbreak by the fastest means of communication possible, such as by telephone or fax. The livestock extension officers working in the sub-districts are also required to report outbreaks of disease to the nearest SVLs/RLDCs, through the District Livestock Officers (DLOs), or directly to the veterinarians in the regional laboratories. A flash report, indicating the suspected disease, date of occurrence, morbidity and mortality and the geographical location of the outbreak, is sent to the nearest SVL/RLDC or even to the NCAH directly. The veterinarians from the SVLs/RLDCs/NCAH then make visits to the herds/villages and undertake a detailed epidemiological and laboratory investigation of the outbreak. Samples, such as sera and vesicular fluid/tissue, are sent to the NCAH for laboratory confirmation. Vesicular tissue samples are then sent to the World Reference Laboratory for FMD (WRLFMD) based at Pirbright, UK for serotyping and molecular analysis. Based on the findings of the epidemiological investigations of the disease outbreaks, technical feedback is given to the livestock offices on the measures to be applied to control the outbreaks.

The LECs/RNRECs are required to submit a monthly animal health report of their jurisdiction sub-district(s) in which information on clinical cases, disease outbreaks, and vaccination are documented. This information is then entered into a Veterinary Information System database (VIS) maintained at the SVLs/RLDCs/NCAH. The laboratories are required to generate monthly reports on the samples tested, diseases diagnosed, and details of outbreak investigations performed. This is then recorded into a Laboratory Systems database (LABSYS). Animal health reports are generated bi-annually by the NCAH using the VIS and LABSYS and distributed to all animal health institutions in the country.

Figure 1.6 Flow chart depicting pathways of the flow of information for disease reporting and feedback system in the current passive surveillance system in Bhutan



Bhutan, being a member of the Office International des Epizooties (OIE), is required to submit animal health reports to the OIE.

Immediate reporting is done for new outbreaks of OIE list A diseases and thereafter weekly reporting is done until the disease outbreak is resolved. Routinely six-monthly and annual reports on the animal health situation are produced for the OIE. The LABSYS and VIS has now been replaced by the TADINFO database system introduced by the Food and Agriculture Organisation (FAO).

Active serological and clinical surveillance are rarely undertaken for FMD owing to a lack of funds and resources.

1.3.2 Foot-and-mouth disease control programme

Foot-and-mouth disease is the most important livestock disease in Bhutan. A control programme had been in place since the early 1960's. However, the control activities were not initially undertaken in an organised manner owing to a lack of veterinary infrastructure, trained persons, and funds. Coinciding with the launch of the European Union funded project on strengthening of veterinary services in the early 1990's, the National Foot-and-mouth Disease Control Programme (NFMDCP) was launched in 1996. The NFMDCP (Anonymous, 1996) focused on undertaking numerous prevention and control activities including prophylactic and post-outbreak vaccination, regulated movements of livestock and livestock products, and awareness programmes for farmers and other stake holders. Disease prevention and control activities were spearheaded by the regional laboratories set up under the project including support for the supply of vaccines. Vaccination, using the tetravalent FMD vaccines (serotypes O, A, Asia 1 and C), was advocated bi-annually in the high risk areas and the country was divided into three zones: epidemic (districts bordering India), endemic (districts with relatively few outbreaks) and sporadic (rare and scattered

occurrence of FMD). There was limited success in the control of FMD during this period (1996-2005) due mainly to a lack of focal coordinating agencies; budgetary constraints; a lack of sufficient cold-chain facilities and equipment to store and transport vaccines; inadequate diagnostic facilities and insufficient epidemiological information (Anonymous, 2005).

The NFMDCP was revised in 2005 (Anonymous, 2005) with emphasis on setting up coordinating focal agencies at various levels. The primary aim of this revised programme was to create buffer zones in the frontier districts through sustained vaccination. The country was divided into three zones (high, medium, and low risk) based on the incidence of outbreaks. However, this was not based on extensive analysis of the epidemiological data available from the passive surveillance system adopted. Emphasis was put on calf-hood vaccination by vaccinating calves at four months of age and revaccinating them one month after the initial vaccination. In the high risk areas, all FMD-susceptible species were to be vaccinated, whereas in the medium and low risk zones, only large ruminants were to be vaccinated. Trivalent vaccines containing serotypes O, A and Asia 1 produced by Intervet® (Pune) and Indian Immunologicals® (Hyderabad) from India, are used routinely. In the high and medium risk areas, prophylactic vaccination was advocated bi-annually, in September/October and March/April. Currently, disease prevention and control is undertaken as per the policy document on the revised FMD control in Bhutan (Anonymous, 2005).

1.4 Aims and Objectives

Prior to this study, there was very limited information available about the epidemiology of FMD in Bhutan. Understanding disease epidemiology, in terms of disease distribution and persistence, risk factors, animal movement patterns, and application of zoning is very

relevant to Bhutan, as in other endemic countries (Rweyemamu *et al.*, 2008b). Unlike many other countries, due to socioeconomic reasons, Bhutan cannot afford to undertake drastic control measures such as ‘stamping-out’ of infected animals to control the disease. Implementing and evaluating a control programme in Bhutan hinges upon a thorough understanding of the disease’s epidemiology.

Therefore, the main aim of this thesis is to generate baseline epidemiological information about FMD in Bhutan to support and strengthen the existing control programme.

The main objectives of this thesis are:

- To describe the spatial and temporal distribution of FMD in the country
- To understand the behaviour of the PanAsia strain of the O serotype under different control situations in Bhutan.
- To identify the risk factors, in sedentary and migratory herds, associated with the occurrence and spread of FMD in the country.
- To validate the passive surveillance system through seroprevalence surveys in sedentary and migratory herds.
- To understand the animal movement patterns in an FMD-endemic and an FMD-free district to support the information on the epidemiology of FMD in Bhutan
- To validate the FMD-free status in Tsirang district in order to initiate progressive zoning approaches as an alternative means for the control of FMD in Bhutan.
- To provide recommendations for further strengthening of the current NFMDCP based on the findings of this study

The thesis uses a combination of retrospective and prospective studies to unravel the distribution, patterns of occurrence, risk factors, and surveillance of FMD in Bhutan.

Chapter 3 analyses the retrospective data, collected through the country’s passive

surveillance system, to understand the disease's spatiotemporal distribution and identify the high risk areas. The epidemiological behaviour of the PanAsia strain of the O serotype under different control scenarios in Bhutan is reported in Chapter 4. A study on the livestock husbandry practices and risk factors for the occurrence and transmission of FMD in the sedentary herds in four districts is presented in Chapter 5. The findings of the farmer-reported surveillance (Chapter 3) and questionnaire-based surveillance (Chapter 5) in four districts are validated using an active serological survey in these districts (Chapter 6). The seroprevalence and risk factors for FMD in the transhumant herders in three districts is presented in Chapter 7 in order to understand the risks associated with the traditional migratory practices in Bhutan. Chapter 8 unravels the livestock trading and animal movement patterns in two districts with differing FMD statuses. Serological and clinical surveillance studies were conducted to validate the FMD-free status in one of the districts in Bhutan based on the country's passive surveillance system (Chapter 9). Chapter 10 discusses the immune status in a vaccinated cattle population in Bhutan. The findings of the thesis are discussed and summarised in Chapter 11 with conclusions and recommendations to further strengthen the existing control programme in Bhutan.

Literature review

2.1 Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease which affects all cloven-hoofed domestic animals including cattle, sheep, goats, pigs and buffalo. The FMD virus has seven clinically indistinguishable serotypes: O, A, Asia 1, SAT 1 (South African Territory), SAT 2, SAT 3 and C. These serotypes do not induce cross protection against each other and therefore vaccination against one serotype will not provide protection against other serotypes. The virus is also known to affect a wide range of cloven-hoofed wild animals including wild pigs, deer, antelopes, although, apart from the African buffalo (*Syncerus caffer*), the other wild species do not seem to play a significant role in the epidemiology of FMD (OIE, 2008). Cattle are the main reservoir hosts for the virus, although in some instances, the virus is found to adapt to pigs, sheep and goats (OIE, 2008). In Africa, the virus is known to be maintained in cattle and in African buffalo and these species are the main hosts for the disease (OIE, 2008).

The disease is endemic in many parts of the world, particularly in developing countries of Asia, Africa, Middle East, and some parts of Europe. Presently very few countries, which include Canada, USA, Australia, New Zealand, Japan, and parts of Europe and South America, are free of FMD.

In Bhutan, FMD is endemic mostly in the southern districts bordering India and in these districts outbreaks of FMD are reported almost every year (Tshering, 2003). The disease is reported sporadically in the interior parts of the country, however periodic epidemics also occur (Tenzin, 2007).

2.2 The FMD virus

The FMD virus (FMDV) is a non-enveloped virus containing a single stranded positive sense-RNA of approximately 8.2 Kb and belongs to the *Aphthovirus* genus of the family *Picornaviridae*. (Kitching *et al.*, 2005). The viral genome has 60 copies of each of four structural proteins (VP1, VP2, VP3 and VP4) (Grubman and Baxt, 2004) and these proteins are derived from the proteolytic cleavage of a single large precursor molecule (Clavijo *et al.*, 2004). These capsid proteins are structural proteins in contrast to the other eight minor proteins (L, 2A, 2B, 2C, 3A, 3B, 3C, and 3D) which are the non-structural proteins (Clavijo *et al.*, 2004, Foord *et al.*, 2007). The virus has a basic organisation similar to that of other members of the *Picornaviridae*. The virus, in common with other RNA viruses, has a high mutation rate during viral RNA replication (Kitching *et al.*, 2005) and this is important in the epidemiology of the disease.

2.2.1 Antigenic variation

Among the animal viruses, FMDV is known to have a very high rate of antigenic variation as evidenced by the presence of seven serotypes and multiple sub-types or strains within these serotypes. Such expression of antigenic variation during the course of an infection is one of the mechanisms to evade the host immune response. This antigenic variation is a result of the very high mutation rate in the viral RNA during replication of the virus, which is in the range of 10^{-3} to 10^{-5} per nucleotide site per genome replication (Drake, 1993, Drake and Holland, 1999) due to the lack of correcting mechanisms during RNA replication. Such high mutation rates result in the replicated FMDV genome being different from their parental strand by 0.1 to 10 base positions (Haydon *et al.*, 2001b). The genetic variants of FMDV are known to accumulate rapidly in the field and co-circulate (Martinez *et al.*, 1992, Samuel *et al.*, 1999). Most of the variation is known to occur within the capsid-coding region of the genome and leads to antigenic variation (Grubman and

Baxt, 2004). The antigenic variation of the FMDV is known to increase with time and this phenomenon is as a result of the immunologic pressure placed on the virus by either vaccinated or infected host species (Haydon *et al.*, 2001a, Domingo *et al.*, 2003). Virus genome sequencing and antigenic variation studies have been invaluable in understanding the molecular epidemiology of the disease in determining the source of outbreaks. The control of this disease by vaccination programmes alone has been largely unsuccessful due to the emergence of new variants (strains) of the virus induced as a result of the immunologic pressure by some of the vaccines (Haydon *et al.*, 2001b).

The determination of nucleotide sequencing of the viral RNA allows unequivocal characterisation of genetic relationships between viral strains (Knowles and Samuel, 2003). The seven serotypes of FMDV are grouped into seven distinct genetic lineages with approximately 30-50% differences in the VP1 gene (Knowles and Samuel, 2003). Based on VP1 nucleotide sequence differences, the O serotype FMDV has been grouped into eight genetically distinct topotypes (Samuel and Knowles, 2001). These topotypes are Cathay, Middle East-South Asia (ME-SA), South-East Asia (SEA), Europe-South America (Euro-SA), Indonesia-1 (ISA-1), Indonesia-2 (ISA-2), East Africa (EA) and West Africa (WA). Two of the topotypes, ISA-1 and ISA-2, have not been recorded outside Indonesia and are now thought to be extinct (Knowles and Samuel, 2003). The O serotypes isolated from Bhutan all fall under the ME-SA topotype (http://www.wrlfmd.org/fmd_genotyping/asia/bhu.html - accessed on 25 April 2011).

2.2.2 Virus survival

Foot-and-mouth disease virus is most stable near a neutral pH. At pH 7.5, the virus may remain infective for 18 weeks at 4°C, 11 days at 20°C, 21 hours at 37°C, 7 hours at 43°C, 1 hour at 49°C, 20 seconds at 55°C, and only 3 seconds at 61°C (Bachrach *et al.*, 1957,

Hyslop, 1970). The virus is rapidly inactivated at a pH below 5 or above 11 (Donaldson, 1987).

The virus can be detected within the musculature of slaughtered animals for up to 30 hours but is destroyed by the formation of lactic acid during *rigor mortis* at 4°C (Donaldson, 1997). However, if the carcass is frozen before the onset of *rigor mortis*, the virus can survive for up to 8 months (Hyslop, 1970). The effect of temperature on the survival of the virus is influenced by the amount of tissue debris present and whether the virus is free or associated with cells (Donaldson, 1997).

The virus can survive for up to 6 months in slurry in winter (Donaldson, 1987, Kitching and Mackay, 1994) but is rapidly inactivated by acids and alkalis, including citric acid, phosphoric acid, sulphuric acid, lactic acid, hydrochloric acid, sodium carbonate, sodium hydroxide and sodium metasilicate. The infectivity of FMDV can be destroyed instantaneously by 1 or 2% sodium hydroxide (Shafiyi, 1968). Calcium hydroxide, when used at a 40% solution at a rate of 40-60 l/m³, inactivated FMDV in liquid manure within 4 days. This compound was also found to be equally potent in inactivating FMDV in manure at temperatures between 0 and -10°C (Haas *et al.*, 1995).

2.2.3 Source of virus

The FMDV is excreted in several of the body secretions and excretions including saliva (Hyslop, 1970), milk (Burrows, 1968a), semen, urine, faeces (Donaldson, 1987), nasal discharges and exhaled air (Donaldson, 1986). The infected animals usually start excreting virus at least 24 hours prior to the development of clinical signs (Sutmoller *et al.*, 2003). This has important epidemiological implications as animals are infectious before the onset of clinical signs. However, the findings of a recent research done at the Institute of Animal Health, UK (Charleston *et al.*, 2011) using the FMDV O serotype strongly indicate that

cattle do not become infectious until, on an average, 0.5 days after clinical signs appear. The level of viral secretion is known to drop within 5 to 7 days after the development of lesions coinciding with the development of antibodies (Graves *et al.*, 1971). The virus is therefore likely to be present in all body fluids, especially during the viraemic phase of the disease. Amongst the domestic animals, pigs are known to be the “amplifier hosts” with a capacity to release 3000 times more virus than cattle or sheep (Sellers *et al.*, 1971b). The amount of virus shed is known to vary depending on the species (Sellers and Parker, 1969), the stage of the disease (Graves *et al.*, 1971), and the infecting strain (Donaldson *et al.*, 1970).

2.3 Pathogenesis and Clinical signs

After entry through the aerosol route, the initial virus replication takes place in the pharyngeal or lung tissue (Burrows *et al.*, 1981, Brown *et al.*, 1992). If the virus has entered through skin abrasions, initial multiplication occurs in the dermal and subdermal tissues. The virus is then distributed, through the vascular system to the predilection sites such as the epithelium of the feet, oro-pharynx, teat and udder (Sutmoller *et al.*, 2003). Virus multiplication at these sites leads to the development of lesions which is usually preceded by fever. The virus also probably replicates in the pituitary gland and this is speculated to be the cause of the “heat intolerance syndrome” or “panting” seen in recovered animals (Domanski and Fitko, 1959, Scott *et al.*, 1965). The clinical signs of FMD are more apparent in FMD-naïve animals than in animals from endemic areas or in an immune population.

2.3.1 Cattle

The incubation period in cattle can range from 2 to 14 days depending on the infecting dose, the virus strain and the route of infection (Donaldson, 1987). Affected animals will

initially exhibit pyrexia, lasting for one or two days, followed by development of vesicles on the tongue, hard palate, dental pad, lips, gums, muzzle, coronary band and interdigital space (Kitching, 2002b). Infected animals have excessive salivation and a nasal discharge. Signs of lameness are exhibited by frequent stamping of the feet. Depending on the severity of the disease and the host susceptibility, lactating animals can develop vesicles on the teats. This makes milking painful and the ruptured vesicles can become infected leading to secondary mastitis. There is a significant drop in milk production. The vesicles in the mouth usually rupture within 24 hours of formation, leaving shallow erosions surrounded by shreds of epithelium (Kitching, 2002b). Healing of oral lesions is usually rapid, in contrast to those on the feet (Kitching, 2002b). The disease morbidity can reach up to 100%, especially in a non-immune population; however, mortality is usually restricted to young animals that can develop viral myocarditis. In adult cattle, mortality is normally below 5% whereas calves can have a mortality of up to 50% (Sard, 1978). Clinical signs are more apparent in cattle as compared to other domestic species (Davies, 2002).

2.3.2 Sheep and goats

The incubation period in sheep and goats ranges from three to eight days, but can vary from as short as 24 hours to as long as 12 days (Kitching and Mackay, 1994). Unlike in cattle, clinical signs are often inapparent in sheep and goats resulting in the disease frequently being missed during routine clinical inspections. The susceptibility of sheep and goats to FMD is also known to depend on the virus strain and the breed of animals (Kitching and Hughes, 2002). Lameness is usually the first indication of FMD in sheep and goats (Kitching and Hughes, 2002).

2.3.3 Pigs

Generally, the incubation period in pigs is 2 to 6 days (Donaldson, 1987), although it depends on the dose and strain of virus, route of infection and individual susceptibility (Kitching and Alexandersen, 2002). Infection with a low dose of virus usually results in sub-clinical disease in pigs with no detectable clinical signs (Kitching and Alexandersen, 2002). In acute cases, infected pigs will show signs of lameness, blanching of the skin around the coronary bands, vesicle formation on the nostrils and in the mouth and pyrexia. Lesions around the coronary bands are the most consistent lesions seen in this species (Kitching and Alexandersen, 2002). The vesicles in the mouth are less conspicuous than in cattle and therefore can be missed during routine examination. The pig-adapted Cathay topotype of O serotype is highly virulent in pigs producing clinical disease within 18 hours of infection (Dunn and Donaldson, 1997, Kitching and Alexandersen, 2002).

2.4 Disease epidemiology

2.4.1 Hosts

Foot-and-mouth disease affects all cloven-hoofed domestic and wild animals (Thomson *et al.*, 2003). The domestic animals most commonly affected include cattle, sheep, goat, pigs, yaks, buffalo (OIE, 2008). Some commonly affected cloven-hoofed wild animals include deer and wild pig (Davies, 2002), African buffalo (*Syncerus caffer*) (Hedger and Condy, 1985) mountain gazelles (*Gazella gazella*) (Shimshony *et al.*, 1986) and mithun (*Bos frontalis*).

Foot-and-mouth disease has also been recorded in other wild animals such as those belonging to Camelidae family including camels, llamas, and alpacas (Wernery and Kaaden, 2004); hedgehog (*Erinaceus* spp.) (McCauley, 1963); and Asian (*Elephas maximus*) and African elephants (*Loxodonta africana*) (Schaftenaar, 2002).

2.4.2 Mechanisms of spread

The most common route of entry of FMDV is through the respiratory tract (Sellers and Parker, 1969, McVicar and Suttmoller, 1976) although, less commonly, the virus can gain entry through abrasions in the epithelium of the oral cavity, feet or teats. However entry via abrasions is very inefficient requiring almost 10,000 times more virus than the respiratory route for infection to occur (Donaldson, 1987). Cattle are the most susceptible species to be infected by the inhalation route owing to their large inspiratory capacity compared to other susceptible domesticated species (Sellers *et al.*, 1971b, Suttmoller *et al.*, 2003).

2.4.2.1 Direct and indirect contact

Foot-and-mouth disease is most commonly spread by direct contact between an infected and a susceptible animal (Kitching *et al.*, 2005). The disease can also be spread indirectly by farmers, veterinarians, inseminators, and movement of contaminated animal products such as meat, milk, semen and hides. Activities such as shearing, de-worming, blood sampling and milking can also lead to indirect contact with virus from infected animals (Alexandersen *et al.*, 2003b) if proper hygiene and aseptic precautions are not taken. Milk trucks were implicated as an important source of virus spread in an outbreak in the UK (Sellers *et al.*, 1971a). Studies in that country showed that imported meat accounted for between 54 and 77% of the 179 primary outbreaks in the UK between 1954 and 1967 (Anonymous, 1969).

Humans have also been known to transmit the disease mechanically to animals as was demonstrated during the outbreak of FMD type A in Canada (Hyslop, 1970) whereby an agricultural worker from Germany introduced the virus into the country. Similarly, veterinarians have also been incriminated in the spread of disease in 6 of the 51 outbreaks during the 1967-1968 epidemic in the UK (Suttmoller *et al.*, 2003).

2.4.2.2 Transmission by ingestion

Animals, especially pigs, can be infected through ingestion of contaminated uncooked swill (Alexandersen *et al.*, 2003b). Recent outbreaks in South Africa in 2000 and the UK in 2001 have been linked to the feeding of unheated waste food to pigs (Knowles *et al.*, 2001). The source for the epidemic in Miyazaki prefecture, Japan in 2000 was linked to feeding of contaminated fodder (wheat straw) to cattle (Knowles *et al.*, 2001, Sugiura *et al.*, 2001). Although oral transmission is possible, significantly higher doses of virus are required by this route as compared to infection by inhalation (Donaldson, 1987).

2.4.2.3 Air-borne transmission

Infected animals produce a large amount of virus in their exhaled air. Cattle and sheep are particularly susceptible to infection by the aerosol route compared with pigs (Kitching *et al.*, 2005). For instance, cattle can be experimentally infected by airborne exposure to as little as 10 tissue culture 50% infective doses (TCID₅₀) as compared to pigs which require more than 10³ TCID₅₀ (Alexandersen *et al.*, 2003b). However pigs excrete more aerosolized virus followed by cattle and sheep (Gloster *et al.*, 1982).

An important mode of spread of FMD has been the air-borne spread, especially in temperate countries where the climate is conducive for the survival of the virus. For instance in the UK, several authors (Tinline, 1970, Gloster *et al.*, 1981, Gloster *et al.*, 2003) have reported disease spread by wind during outbreaks. The integration of epidemiological data with meteorological information led to the development of aerosol dispersal models which were used to predict and analyse the airborne spread of FMD in the UK (Gloster *et al.*, 1981, Donaldson *et al.*, 1982, Sutmoller *et al.*, 2003). Relative humidity is known to have the greatest effect on the survival of the FMDV and a relative humidity of 60% or above (Donaldson, 1972) is known to allow the virus to retain maximum infectivity. Cool temperatures and neutral or slightly alkaline conditions are also known to

prolong the survival of virus in infective aerosols and on fomites (Sellers *et al.*, 1971b). The amount of airborne virus released via air movement is dependent on the virus strain, the species of animal affected and the stage of disease. Once released, the virus is known to form “virus plumes” in the air and this is subject to dispersion both horizontally and vertically (Donaldson, 1972).

Conditions such as high virus output, high virus survival and exposure of large numbers of susceptible animals favour the airborne spread of FMDV (Gloster *et al.*, 1982). Several studies in the UK have reported the likely spread of FMD by airborne transport up to 60 km over land and up to 300 km over sea (Gloster *et al.*, 1981, Donaldson *et al.*, 1982, Sørensen *et al.*, 2000). The long distance spread of virus over sea, as compared to land, could be attributed to the relatively constant temperature over the sea (due to a flatter surface) and the stable air conditions which enables the virus to remain suspended for longer periods (Gloster *et al.*, 1982). In the tropics, aerosol spread is not believed to be important given the intense sunlight and high temperatures that could rapidly inactivate the infected aerosols as observed in countries such as Thailand (Chamnanpood *et al.*, 1995, Wongsathapornchai *et al.*, 2008b) and Saudi Arabia (Hutber and Kitching, 2000).

2.4.2.4 Sexual transmission

Foot-and-mouth disease has been experimentally transmitted from infected bulls to heifers through artificial insemination (Cottral *et al.*, 1968). The possibility of sexual transmission of FMDV from carrier African buffalo (*Syncerus caffer*) to cattle has been speculated by Bastos *et al.* (1999) although the claim could not be established unequivocally.

2.4.2.5 Iatrogenic transmission

Use of FMDV-contaminated instruments, such as hypodermic needles during parenteral administration of drugs or vaccines, could lead to transmission of infection (Alexandersen

et al., 2003b). Similarly, use of FMD vaccines containing live virus as a consequence of using inappropriate inactivants or incomplete inactivation processes can also result in infection (Beck and Strohmaier, 1987).

2.4.3 The Carrier problem – persistent infection

Foot-and-Mouth Disease is known to result in persistent virus infection in some species of domestic animals such as cattle, sheep and goats. The carrier state occurs as a low-level infection of susceptible animals whereby there is intermittent excretion of live virus in the oesophageal-pharyngeal secretions (OP) beyond 28 days post-infection (Salt, 1993, Alexandersen *et al.*, 2002). The first demonstration of the presence of infectious viral materials in the OP fluids of convalescent cattle was shown by van Bekkuni *et al.* (1959).

The carrier animals are known to harbour live virus for a considerable period of time (Salt, 1993). Vaccinated animals that come in contact with FMDV can also become carriers. The predilection site for the FMDV is the epithelium of the OP tissue (Burrows, 1966) which makes it the most suitable site for isolation of virus for identification of carriers through the collection of probang samples.

The carrier status is species and virus-strain dependent (Salt, 1993). For instance, cattle (Hedger, 1968) are known to remain as carriers for up to three years; sheep for up to 9 months (Burrows, 1968b, Pay, 1988); and goats for up to 4 months (Alexandersen *et al.*, 2002). Pigs do not become carriers as they clear the infection within 3 to 4 weeks of infection (Alexandersen *et al.*, 2003a). In wildlife, it has been well established that the African buffalo (*Syncerus caffer*) can remain a carrier for up to 5 years (Condy *et al.*, 1985). Other wildlife species such as impala, warthog and bush pig are not known to remain as carriers (Hedger *et al.*, 1972). In Africa, it has been shown that all serotypes of SAT viruses are found in the wild African buffalo populations of Botswana and

Zimbabwe. In contrast these serotypes are not maintained in cattle, sheep or goats (Kitching, 2002a).

2.4.3.1 Role of carriers in disease transmission

The role of carriers in precipitating new outbreaks and aiding in disease transmission is still a debatable issue (Sangare *et al.*, 2004). There is anecdotal evidence from the field suggesting that carriers can start new outbreaks of FMD (Salt, 1993). The strongest evidence for this comes from the Zimbabwean outbreaks of 1989 and 1991 when carrier African buffalo were found to have transmitted infection to cattle (Dawe *et al.*, 1994). It has been suggested that movement of carrier animals into new locations can trigger outbreaks, as the stress of movement could depress their immunity and precipitate shedding of virus (Hedger and Condy, 1985). However, so far, it has not been possible to confirm the role of carrier cattle as a potential source of new outbreaks, both in the field and experimentally (Alexandersen *et al.*, 2002, Sutmoller *et al.*, 2003). The role of carrier sheep and goats is confusing with some authors (Anderson *et al.*, 1976, Parida *et al.*, 2008) suggesting that these animals play an insignificant role in disease transmission while others (Sharma, 1981, Hancock and Prado, 1993) refute this claim. Therefore, the significance of the carrier state for FMD is still not fully understood (Kitching *et al.*, 2007).

2.4.3.2 Identification of carriers

The prevalence of carriers in a population depends on the species, herd immunity, and amount of disease in a population (Alexandersen *et al.*, 2002). In endemic areas, it is not unusual to come across carrier animals. For instance in Asiatic Turkey (Gurhan *et al.*, 1993), about 15-20% of infected cattle and sheep were found to be carriers and similar surveys have detected up to 50% of carriers in Brazil (Sutmoller and Gaggero, 1965).

Diagnostic polymerase chain reaction (PCR) testing (House and Meyer, 1993, Donn *et al.*, 1994) has been used widely to detect viral genomes in OP fluids from carrier animals. The other, more cost-effective, way of detecting carriers is through the use of serological tests. In a non-vaccinated population, serological tests based on structural and non-structural FMDV proteins (NSPs) can be used to detect carriers. However, in a vaccinated population, conventional structural protein tests cannot distinguish antibody induced by natural infection from that caused by vaccination. Therefore serological tests based on NSPs are an important tool to detect anti-NSP antibodies (Kitching, 2002a) present in carrier animals. However, given the limitations of the current NSP tests and the lack of purity of the FMD vaccines, the tests cannot be used at an individual animal level to identify carrier animals. For instance, cattle that have been vaccinated with a potent vaccine may fail to develop antibodies to NSPs should they have contact with live virus and undergo some mucosal infection (Kitching, 2002a). On the contrary, presence of residual NSPs in some of the commercial FMD vaccines can elicit development of anti-NSP antibodies in vaccinated animals and therefore result in false positive reactions (Lee *et al.*, 2006, Chen *et al.*, 2008).

2.4.4 Geographical distribution of FMDV serotypes

There are seven serotypes of FMDV which produce clinically-indistinguishable disease in affected animals. The distribution of the disease is dependent on the distribution of the serotypes. For instance, the three SAT serotypes, SAT 1, SAT 2, and SAT 3 are generally restricted in distribution to Africa (Knowles *et al.*, 2005, Kitching *et al.*, 2007), although some of these have caused sporadic outbreaks in Saudi Arabia and Kuwait (Aidaros, 2002).

The most predominant serotype is serotype O which has been virtually reported from all FMD-affected countries. The recent pandemic strain of the serotype O, the PanAsia strain, belongs to the ME-SA topotype. This strain has caused significant outbreaks in Asia, and continues to be a major threat to Europe and other FMD-free countries (Klein, 2009). Some strains of serotype O are known to be species-specific and include the pig-adapted Cathay topotype (Cheng *et al.*, 2006). However, the PanAsia strain is not species-specific (Knowles *et al.*, 2005).

Serotype A is known to have the most antigenic diversity and there often is no cross-protection between strains (Bronsvort *et al.*, 2004b, Klein *et al.*, 2006) within serotype A. The Asia 1 serotype is mostly restricted to Asia and has the least antigenic and genetic variability amongst the seven serotypes (Sanyal *et al.*, 2004, Valarcher *et al.*, 2005). This serotype is rarely reported outside Asia, although there was a brief incursion into Turkey and Greece in 2000 (Valarcher *et al.*, 2005, Kitching *et al.*, 2007).

Apart from causing few sporadic outbreaks in South America, East Africa and Pakistan between 2000 and 2006, serotype C has been characterised by a long absence from the circulating virus pool (Kitching *et al.*, 2007, Klein, 2009). Type C FMDV has not been detected in South, South-East, or East Asia for a long time and now it is believed that the serotype is no longer circulating in the region (Rweyemamu *et al.*, 2008b).

2.4.5 Disease status in Asia

Only four serotypes (O, A, C and Asia 1) have been recorded in Asia (Rweyemamu *et al.*, 2008b).

2.4.5.1 South-East Asia

In South-East Asia (SEA), the disease is endemic in Cambodia, Lao PDR, Myanmar, Thailand, mainland Malaysia, and Vietnam. Brunei, Indonesia, Malaysia (zone covering

the provinces of Sabah and Sarawak), Singapore and the Philippines (one zone consisting of the Mindanao Islands; one zone consisting of the Islands of Visayas and the provinces of Palawan and Masbate; and three separate zones that cover the whole Island of Luzon) are currently free from FMD (OIE, 2011).

In a review of the period from 1996 to 2001 (Gleeson, 2002), serotype O reportedly caused outbreaks in all seven FMD-endemic countries in SEA. Three different type O lineages have been recorded, namely, the South-east Asian topotype; the pig adapted or Cathay topotype and the PanAsian topotype (Gleeson, 2002). In general terms, O, A and Asia 1 were the three serotypes endemic in the region from 1996 to 2001 (Gleeson, 2002) and between 2001-2005 (Abila and Foreman, 2006).

2.4.5.2 South Asia

In South Asia, FMD is endemic in all countries including India, Pakistan, Nepal, Bhutan, Sri Lanka, Bangladesh and the Maldives (OIE, 2011). The disease is endemic in India (Khera and Mukopadhyay, 1985, Venkatesh *et al.*, 2008, Saravanan *et al.*, 2009) and a review revealed that four serotypes, O, A, C and Asia 1 were prevalent in India from 1976-1984 (Khera and Mukopadhyay, 1985). However, reviews from recent outbreaks show that serotypes O, A and Asia 1 are most commonly isolated (Sarma and Sutopa, 2003, Verma *et al.*, 2008). Of these, serotype O is the one most commonly recorded in India (Sarma and Sutopa, 2003, Thakur *et al.*, 2007, Verma *et al.*, 2008). Similar findings have been reported from other South Asian countries including Bangladesh (Islam *et al.*, 2001), Bhutan (Tshering, 2002) and Nepal (Gongol, 1998) where serotype O is the most commonly recorded serotype.

In general, there is lack of epidemiological data, especially on the spatiotemporal distribution of topotypes in South Asia (Rweyemamu *et al.*, 2008b). There is also a general

lack of data on the dynamics and impact of FMD in South-East Asia (Gleeson, 2002, Perry *et al.*, 2002).

2.4.6 Disease status in the rest of the world

Currently countries such as USA, Canada, Australia, New Zealand, most countries in Europe, and Uruguay are free of FMD with or without vaccination (OIE, 2011). Some countries in South America (Argentina, Brazil, Bolivia, Colombia and Paraguay), and Africa (Botswana) have FMD free zones, either with or without vaccination (OIE, 2011).

2.4.7 The PanAsia Strain of O serotype

A particular genetic lineage of serotype O, referred to as the PanAsia strain, was responsible for an explosive pandemic in Asia which extended to parts of Africa (South Africa only) and Europe from 1998 to 2001 (Knowles *et al.*, 2005). Retrospective examination of viruses from India indicated that the PanAsia strain was present in the north of that country as early as 1990 and may even have been present as far back as 1982 (Hemadri *et al.*, 2002). The PanAsia strain, after several years in India, spread through Southern Asia, the Middle East, and finally into Europe and South Africa (Knowles *et al.*, 2005). This strain was detected in samples submitted from Nepal in 1993 and 1994 and from Bhutan in 1998 (Knowles and Samuel, 2003). The PanAsia strain was first detected in Bahrain, Iran, Lebanon, Kuwait, Saudi Arabia, and Yemen in 1998; in Israel, Turkey, and The United Arab Emirates in 1999; and in Malaysia in 2000 (Knowles *et al.*, 2005). Similarly, the PanAsia strain was found to be involved in the outbreaks that occurred in China in 1999; all mainland South-East Asian countries in 1999 and 2000; South Korea, Japan, Russia, Mongolia, and South Africa in 2000; and in the UK, Northern Ireland, Netherlands and France in 2001 (Knowles *et al.*, 2005).

The PanAsia virus strain has been isolated from a wide variety of hosts including cattle, water buffalo, pigs, sheep, goats, and gazelle (Knowles *et al.*, 2005).

In Bhutan, the PanAsia strain of the O serotype was detected for the first time in 1998 in samples submitted to the World Reference Laboratory for FMD (WRLFMD) in Pirbright, UK (Knowles and Samuel, 2003). Thereafter, the strain was detected in the outbreaks of 2002, 2003 and 2004 (http://www.wrlfmd.org/fmd_genotyping/asia/bhu.htm., accessed on 4th November 2009). There are no records for the years 2005 and 2006 since no samples were sent to the WRLFMD. In the 2007 outbreak, a new PanAsia lineage (PanAsia-2) was detected in the samples submitted to the WRLFMD. PanAsia-2 strain was again detected in samples from an outbreak in 2008. So far the PanAsia (Pandemic) strain has not been detected in Africa (except South Africa) and South America (Knowles *et al.*, 2005).

2.4.8 Risk factors for FMD

The greatest risk for the spread of FMD comes from the movement of infected live animals. The 2001 FMD epidemic in the UK highlighted the role of the movement of infected animals where infected sheep, undetected due to the sub-clinical nature of the disease in this species, spread virus to other parts of the country before the first case was diagnosed (Gibbens *et al.*, 2001). The source of the 2001 outbreak in the Netherlands was traced to the importation of infected calves from the UK (Pluimers *et al.*, 2002). Animal movements have also been cited as one of the most common methods of spread of FMD in South-East Asia (Perry *et al.*, 2002, Abila and Foreman, 2006, Wongsathapornchai *et al.*, 2008a) and other parts of the world (Perez *et al.*, 2004, Christley *et al.*, 2005).

In countries where cattle farming is semi-intensive and subsistence-orientated, mixing of animals at grazing and watering areas is an important risk factor for the spread of FMD as was shown in studies in Thailand (Cleland *et al.*, 1996, Rojanasthien *et al.*, 2006),

Cambodia (Sothyra, 2008), Myanmar (Oo, 2010), and Cameroon (Bronsvort *et al.*, 2004a).

Studies on disease risk factors have identified many other factors linked with the presence and spread of FMD, such as owning sheep and goats along with cattle (Megersa *et al.*, 2008), mixed farming of sheep and goats (Al-Majali *et al.*, 2008), owning buffalo and going on transhumant migration (Bronsvort *et al.*, 2004a), purchase of livestock at markets (Lindholm *et al.*, 2007), and proximity to slaughter houses (Rojanasthien *et al.*, 2006).

2.5 Immunity to FMD

Following infection or vaccination, a rapid humoral response is elicited in the host which is serotype-specific (Grubman and Baxt, 2004). The level of protection offered by this humoral immunity is generally correlated with the level of neutralizing antibodies (McCullough *et al.*, 1992, Salt, 1993).

2.5.1 Immunity following infection

Following infection, immunity to FMD is mediated by the circulating antibodies and titres of these antibodies indicate the level of protection in the convalescent animals (Alexandersen *et al.*, 2003b). Significant titres of circulating antibodies, largely IgM-mediated, can be detected as early as 3-5 days post-infection (pi) (Salt, 1993) and peaks at around 10-14 days before declining to low levels within 30-40 days (Brown *et al.*, 1964). Thereafter, isotype-switching occurs and serotype-specific IgG₁ antibodies can be detected as early as 7-10 days pi (Salt, 1993). Measurable levels of IgG₂ and IgA can also be detected in infected animals at 7-10 days pi although IgG₁ is known to dominate over the other two immunoglobulins (Salt, 1993, Salt *et al.*, 1996).

Serum antibody levels can remain at protective titres for prolonged periods for up to 4.5 years in convalescent cattle (Cunliffe, 1964, Salt, 1993).

2.5.2 Immunity following vaccination

Following vaccination, humoral immunity is elicited with development of IgM antibodies as early as 2 to 4 days post-vaccination (Abu Elzein and Crowther, 1981). The time taken for build up of protective immunity in vaccinated animals depends on the type and quality of vaccine used. Although maximum antibody titres are typically reached 7 to 10 days post-vaccination, protective titres are reached after only 2 to 3 days post-vaccination (Woolhouse *et al.*, 1996). Vaccinated cattle are considered protected if the antibody titres exceed 100 by the LPBE (liquid phase blocking ELISA) (Hamblin *et al.*, 1987, Periolo *et al.*, 1993). The protective immunity provided by vaccination is relatively short lived in comparison to natural infection, and usually lasts only between three to six months, following a single vaccination (Salt, 1993). However, repeated vaccination has been found to result in high titres of antibody in cattle, up to four (Dekker and Terpstra, 1996) and six (Remond *et al.*, 2001) years after the cessation of vaccination.

2.5.3 Maternal Immunity

Newborn animals derive maternal immunity from their vaccinated dams through the ingestion of colostrum (Graves, 1963). Presence of colostral-derived antibodies can hinder the development of immunity (Nicholls *et al.*, 1984). Maternally derived antibodies can be detected within 24 hours following ingestion of colostrum and can be detected up to 6 months of age in cattle (OIE, 2009a).

2.6 Diagnosis

Although FMD leads to the development of characteristic vesicular lesions in affected animals, these are not clinically distinguishable from other vesicular diseases such as

Vesicular Stomatitis, Vesicular Exanthema, and Swine Vesicular Disease (Geering and Lubroth, 2002). Therefore, confirmatory laboratory diagnosis is required to differentiate these vesicular diseases. In FMD-free countries, because the animals are naïve, infected animals show clear clinical signs of the disease. On the contrary, in endemic countries, where vaccination is used, the clinical signs may not be apparent due to either natural or vaccinal immunity.

2.6.1 Clinical diagnosis

The lesions of FMD are characteristic of a vesicular disease and consist of vesicles on the feet, in and around the mouth and on the mammary glands and teats. However, there are interspecies variations in the susceptibility to FMD. Cattle are the most susceptible of the domestic species and hence clinical signs are more obvious in this species (Davies, 2002). Dairy cattle may develop mastitis as a sequel to FMD. Pigs are quite susceptible and show varying severity of clinical signs even to the point of shedding their hooves in severe infections of the feet (Kitching and Alexandersen, 2002). Sheep and goats do not usually show obvious clinical signs and hence clinical diagnosis in this species is generally not conclusive (Geering, 1967, Sharma, 1981, Pay, 1988).

Under field conditions, FMD is usually diagnosed based on the presence of clinical signs and other epidemiological features such as the rapid spread of disease in a herd/village and the associated low mortality.

2.6.2 Laboratory diagnosis

As FMD is clinically indistinguishable from other vesicular diseases confirmation of the disease involves use of laboratory tests (OIE, 2008). A range of laboratory diagnostic tests are used to detect antigen and antibody in infected animals. The disease can be diagnosed by virus isolation or by demonstration of FMD viral antigen or nucleic acid in samples of

tissue or fluid (OIE, 2008). Presence of virus-specific antibodies can be used for diagnosis and antibodies to viral non-structural proteins can indicate infection, irrespective of vaccination status (OIE, 2008).

2.6.2.1 Virus detection tests

These tests detect the presence of FMDV in samples such as epithelium, OP scrapings, serum and cell culture supernatants using methods such as virus isolation with neutralisation of FMDV infectivity by serotype specific antiserum, antigen detection ELISA and reverse transcriptase polymerase chain reaction (RT-PCR) (OIE, 2008).

a) Virus isolation

The principle behind this technique is to retrieve live virus from samples such as the epithelium of ruptured vesicles in the mouth or feet, OP fluids, and tissues. Suspensions of the samples are inoculated onto cell cultures or into unweaned mice. The primary cell culture system used for isolation of FMDV include primary bovine (calf) thyroid cells and primary pig, calf or lamb kidney cells (OIE, 2008). Established cell lines such as BHK-21 (baby hamster kidney) and IB-RS-2 cells are found to be less sensitive than the previously mentioned primary cells for detecting low amounts of virus (Clarke and Spier, 1980). The cell cultures should be examined for cytopathic effects after 48 hours of inoculation. Once the cytopathic effect is complete, the fluid can then be used in other diagnostic tests to confirm the presence of virus by virus neutralisation tests using serotype or strain specific reference antisera in cell culture, viral antigens by immunological tests, or viral genome by molecular tests on these samples.

b) Immunological tests for viral antigens

Enzyme-linked immunosorbent assays (ELISA) have been used both for the detection of viral antigen and the identification of serotypes. An indirect sandwich ELISA is now routinely used for the diagnosis of FMD (Ferris and Dawson, 1988). In general, complement fixation tests (CFT) have now been replaced by ELISAs because of the former's poor sensitivity, relatively complex procedures and difficulty in interpreting results in some tests because of pro- and anti-complementary factors (Ferris and Dawson, 1988). The CFT produces more cross-reactions than the ELISA and its sensitivity is found to depend on the quality of the samples (Hamblin *et al.*, 1984).

c) Molecular techniques

The conventional diagnostic tests for FMD are not without problems. Although ELISA results can be obtained within a couple of hours, a negative ELISA result doesn't necessarily indicate that the animal is truly negative. In samples where the virus concentration is low, the ELISAs may not be sensitive enough to detect virus (Reid *et al.*, 2001b). The virus isolation methods, though circumventing the problem of poor sensitivity of the ELISAs, still take at least 3 to 4 days to complete. Thus, these tests are not suitable for use in situations where a rapid diagnosis is required for the implementation of effective control measures.

Therefore, molecular techniques, detecting viral nucleic acid, have been developed for rapid and effective diagnosis of FMD. Reverse transcriptase polymerase chain reactions (RT-PCR) have been used for the rapid diagnosis of FMD by amplifying the viral genome in diagnostic materials such as epithelium, milk, serum and OP samples (Amaral-Doel *et al.*, 1993). RT-PCRs have also been used successfully for the detection of FMDV in asymptomatic animals (Marquardt *et al.*, 1995), thereby highlighting its use in detecting

infected animals in the early stages of the disease. However, conventional RT-PCRs are not more sensitive or specific than ELISA or virus isolation methods (Reid *et al.*, 1998) and have to be used in conjunction with these procedures for an accurate diagnosis.

Real-time PCRs have been developed to refine the existing RT-PCRs in order to improve the diagnostic sensitivity and specificity and have now been found to be as sensitive as virus isolation methods (Reid *et al.*, 2001b). Automated real-time PCRs have also been developed (Reid *et al.*, 2003) so that more samples can be examined quickly and with minimal risk of contamination.

(d) Pen-side tests

Rapid diagnosis of highly infectious diseases such as FMD would enable decision makers to rapidly implement disease control measures so as to prevent further spread of disease. A rapid pen-side test, based on chromatographic strip test technology, has been developed using both field and experimental samples (Reid *et al.*, 2001a). The test was found to be more sensitive than the ELISA for the diagnosis of all seven serotypes of FMDV in epithelial suspensions and nasal swabs and had a sensitivity equivalent to the ELISA for the detection of contemporary virus strains in cell culture supernatant fluids (Reid *et al.*, 2001a). Recently, a new pen-side test, using a FMDV pan-reactive monoclonal antibody that could detect all seven serotypes, has been developed and validated (Ferris *et al.*, 2009). The test produced a diagnostic sensitivity of 84% and specificity of 99%, which was equivalent to that of the existing antigen ELISA.

2.6.2.2 Serological or antibody detection tests

The principle behind these tests is the detection of antibody which has developed against the FMDV in affected animals (OIE, 2008). Serological tests detect antibodies to either

structural proteins (SP) or to non-structural proteins (NSPs) of the FMDV. The tests are conducted to: confirm virus exposure in suspected cases of FMD; certify FMD antibody status in individual animals before export/import; substantiate disease freedom; and to evaluate vaccination efficacy (OIE, 2008).

(a) Structural protein (SP) tests

The SP tests are serotype-specific and are based on the detection of antibodies which develop against the structural proteins during infection or vaccination (OIE, 2008). The SP tests currently in use are the virus neutralization test (VNT), solid-phase competition ELISA (Mackay *et al.*, 2001, Paiba *et al.*, 2004) and LPBE (Hamblin *et al.*, 1986a, Hamblin *et al.*, 1986b). These tests are the prescribed tests for trade and for confirming infection in non-vaccinated animals, as well as for monitoring of immunity in vaccinated animals (OIE, 2008). The ELISA tests are blocking- or competition-based assays that use serotype-specific polyclonal or monoclonal antibodies and are quicker to perform than the VNT (OIE, 2008).

Virus Neutralisation test

The VNT is the OIE-prescribed test for international trade and is the “gold-standard” test against which all current tests are validated. The VNT requires cell culture facilities, the use of live virus and takes between 2-3 days to complete. However, the VNT cannot distinguish between antibodies induced by natural infection with those induced from vaccination (Moonen *et al.*, 2004b).

Liquid phase blocking ELISA (LPBE)

The LPBE is serotype-specific and highly sensitive providing the virus used as antigen closely matches the virus circulating in the field (OIE, 2008). The LPBE is based on

specific blocking of the FMDV antigen in liquid phase by antibodies in the test serum sample (Hamblin *et al.*, 1986a, Hamblin *et al.*, 1986b). Rabbit antisera, specific for the different serotypes of FMDV, are passively adsorbed to polystyrene microwells. After the test serum is allowed to react with the specific FMDV antigen, the test serum/antigen mixture is then transferred to an ELISA plate coated with FMDV trapping antibodies. The presence of antibodies to FMDV in the serum sample will result in the formation of immune complexes and consequently reduces the amount of free antigen trapped by the immobilized rabbit antisera. In turn, fewer guinea pig anti-FMDV detecting antibodies will react in the next incubation step. After the addition of enzyme-labeled (Horse Radish Peroxidase) anti-guinea pig immunoglobulin conjugate and substrate/chromogen solution, a reduction in colour development will be observed, when compared to controls containing free antigen only. However the LPBE is known to have low specificity and is not a robust test, requiring high skills and training to produce reproducible results (Mackay *et al.*, 2001).

Solid Phase Competition ELISA (SPCE)

In order to circumvent the problems with the LPBE, a solid phase competition ELISA (SPCE), using the same polyclonal antisera and purified 146S antigens of FMDV used for LPBE, was developed (Mackay *et al.*, 2001). The SPCE was found to be as sensitive as the LPBE and had a specificity almost equivalent to the VNT. The SPCE is also more robust and easier to use than the LPBE (Mackay *et al.*, 2001).

(b) Non-structural protein tests

One of the drawbacks of the SP tests is the inability to distinguish antibody induced by vaccination with that naturally developing from field infection (OIE, 2008). This is due to

the fact that antibodies to SPs can be induced by viral antigens present in vaccines, as well as in natural infection with virus. The current FMD vaccines consist of chemical-inactivated whole viral antigen combined with saponin, oil, or aluminium hydroxide depending upon the species in which the vaccine is to be used (Fowler *et al.*, 2008). The current FMD vaccines are largely expected to contain purified preparations of inactivated virions and therefore are expected to induce antibody development almost exclusively towards the structural proteins (Mackay *et al.*, 1998). Therefore, the principle of the NSP tests is to detect antibody developed against the non-structural or non-capsid proteins of the FMDV to identify past or current infection, irrespective of the vaccination status of the animals. A number of NSP assays based on radio-immunoprecipitation (Berger *et al.*, 1990), enzyme-linked immunoelectrotransfer blot (Bergmann *et al.*, 1993), or ELISA (Mackay *et al.*, 1998) have been developed for differentiating natural infection from vaccination.

The assays are based on the detection of antibodies to NSPs using antigens produced by recombinant techniques in a variety of *in-vitro* expression systems (OIE, 2008). It is generally agreed that the antibodies to polyproteins 3AB or 3ABC are considered the most reliable indicators of infection with FMDV (Rodriguez *et al.*, 1994, Mackay *et al.*, 1998). Most of the NSP tests rely on polyclonal or monoclonal hybridoma derived antibody reagents (Diego *et al.*, 1997, Mackay *et al.*, 1998, Sørensen *et al.*, 1998), although recombinant antibodies from bacterial expression system (Foord *et al.*, 2007) have been successfully produced and used as well.

Some NSP tests, however, are prone to produce false-negative results as was shown experimentally when the NSP test failed to detect infected cattle which had been vaccinated and subsequently challenged with live virus (Brocchi *et al.*, 2006). The presence of residual NSPs in some commercial vaccines can produce false-positives to the

NSP ELISA's in repeatedly vaccinated cattle (Lee *et al.*, 2006, OIE, 2008) and pigs (Chen *et al.*, 2008).

The antibody response to the NSPs is found to be highly variable across individuals, species of animals, and types of NSPs used. For instance in cattle (Bergmann *et al.*, 1993, Sørensen *et al.*, 1998) the antibody response to 3A, 3B, 3D and 3ABC can be detected as early as 7 to 10 days post-infection. Whereas in sheep, antibodies to NSPs could be detected between 14 and 22 days of infection. There is also variation in the duration of the seropositivity. Anti-NSP antibodies, especially, the anti-3ABC antibodies can be detected in infected animals up to 365 (Diego *et al.*, 1997), 395 (Sørensen *et al.*, 1998), 560 (Silberstein *et al.*, 1997), 570 (Robiolo *et al.*, 2006), and 665 days (Moonen *et al.*, 2004b) after infection.

The OIE Index test for NSP-ELISA is the Panaftosa method (NCPanaftosa ELISA), which is an indirect ELISA using *Escherichia coli* expressed recombinant 3ABC NSPs (OIE, 2008). A number of NSP assays have been developed over the years, using indirect (Diego *et al.*, 1997, Malirat *et al.*, 1998, Shen *et al.*, 1999, Bruderer *et al.*, 2004) or blocking (Chung *et al.*, 2002, Sørensen *et al.*, 2005) ELISAs with recombinant antigens expressed in either *E.coli* or Baculovirus or antigens manufactured as peptides.

Non-structural protein tests are currently the most sensitive tools to detect present or past infection with FMDV in vaccinated cattle (Brocchi *et al.*, 2006). However, the available NSP assays are being evaluated to check their applicability in a variety of situations. These assays are being continually evaluated based on dichotomous results (Brocchi *et al.*, 2006), Bayesian methods (Engel *et al.*, 2008) and analysis of ROC curves (Moonen *et al.*, 2004b, Dekker *et al.*, 2008). Much of the evaluation work has been done in cattle (Brocchi *et al.*, 2006) as compared with sheep (Armstrong *et al.*, 2005) and pigs (Chen *et al.*, 2007). In a number of evaluation studies conducted at various locations, the commercial NSP assay

manufactured by CEDI Diagnostics BV, Lelystad, the Netherlands, and subsequently renamed as PrioCHECK® FMDV NS (<http://www.prionics.com>) has been found to be one of the best amongst the currently available commercial NSP kits. It is a blocking ELISA based on Baculovirus-expressed FMDV 3ABC NSP as antigen and monoclonal antibody against FMDV 3ABC NSP as capture and detector antibody (Sørensen *et al.*, 2005). This kit is reported to have specificities of up to 99.5% in naïve sheep and 99.7% and 99.5% in multiple vaccinated cattle and pigs respectively (Sørensen *et al.*, 2005). The kit can be used in bovine, ovine, caprine and porcine species.

In a comprehensive evaluation based on a panel of a large number of cattle sera from both experimental trials as well as field outbreaks, the PrioCHECK® FMDV NS had an overall specificity of 98.1% (97.2% in non-vaccinated and 99.5% in vaccinated), equivalent to or greater than the OIE-index test *NCPanaftosa* (Brocchi *et al.*, 2006). The kit also was consistently better than three other commercial NSP kits in terms of its ability to detect carriers between 28 to 100 days-post-infection with a sensitivity of 86.4% compared to 93.9% with the *NCPanaftosa*. Based on the criteria that serum was considered as true-positive if at least four of the six tests were positive, the PrioCHECK® had a relative sensitivity of 97.2% as compared to 99.7% for the *NCPanaftosa*. Others (Dekker *et al.*, 2003, Dekker *et al.*, 2008, Engel *et al.*, 2008, Parida *et al.*, 2008) have also found PrioCHECK® to have a higher diagnostic sensitivity and specificity compared with other commercially available kits used in cattle. When evaluated in a population free of FMD in New Zealand, the PrioCHECK had a diagnostic specificity of 99.5% (95% CI: 98.4, 99.7), 99.7% (95% CI: 98.8, 99.8), and 99.6% (95% CI: 97.2, 99.6) in unvaccinated cattle, sheep and pigs, respectively (Kittelberger *et al.*, 2008). The PrioCHECK® has also been evaluated in sheep (Brocchi *et al.*, 2006), pigs (Brocchi *et al.*, 2006, Chen *et al.*, 2007) and buffalo (Bronsvoort *et al.*, 2008). So far no evaluation has been done in goats. In non-

infected sheep (vaccinated and non-vaccinated), the PrioCHECK® (Brocchi *et al.*, 2006) had a specificity of 100% compared to the 98% with the *NCPanaftosa* test.

Brocchi *et al.* (2006) also found that the PrioCHECK® (66.6% Sensitivity) had a better detection rate than the OIE Index test (50%) in vaccinated and experimentally-infected sheep. In the same study, Brocchi *et al.* (2006) found that PrioCHECK® had a specificity of 100% in non-infected pigs (vaccinated and non-vaccinated) and a sensitivity of 100% in non-vaccinated, challenge infected pigs. Similar reports of the superior performance of the PrioCHECK® in pigs, when compared with other commercial kits, have been reported in the literature (Chen *et al.*, 2007).

Other commercial ELISAs available include SVANOVIR® FMDV 3ABC-Ab ELISA (Svanova, Upsala, Sweden; (Persson *et al.*, 2004)), CHEKIT-FMD-3ABC (Bommeli Diagnostics, Bern, Switzerland; (Bruderer *et al.*, 2004)), and UBI® FMDV NS ELISA (United Biomedical Inc., New York, USA; (Shen *et al.*, 1999)). All three kits are indirect ELISAs and use anti-species conjugates. While SVANOVA® and CHEKIT® detect antibodies to the viral non-structural polypeptide 3ABC, the UBI® detects antibodies to a 3B synthetic peptide. The SVANOVA® can be used only in bovines; the UBI® in bovine and porcine; and the CHEKIT® can be used in bovine, ovine and porcine species.

The CHEKIT® had a diagnostic specificity of 99.9%, 99.7% and 99.6% in cattle, sheep and pigs when evaluated in a population in New Zealand where no FMD vaccine was used (Kittelberger *et al.*, 2008). The CHEKIT® had superior diagnostic specificity of 98% than an in-house competitive 3ABC ELISA (90% specificity) developed in Denmark when evaluated using sera collected from an FMD-endemic area (Bronsvort *et al.*, 2004c). Bruderer *et al.* (2004) also reported a specificity of more than 99% in bovine, ovine and porcine species for the CHEKIT® when evaluating sera from FMD-free countries.

Using sera from naïve and vaccinated animals (for specificity) and from experimentally and field infected animals (for sensitivity), the c-ELISA from AAHL, Geelong had a diagnostic sensitivity of 91.48% and a specificity of 96.36% in naïve and 98.02% in vaccinated cattle (Colling *et al.*, 2010) .

Although the NSP tests are currently the only OIE-approved tests for the differentiation of vaccinated from infected animals, the tests can only be applied at the herd level (Clavijo *et al.*, 2004, Paton *et al.*, 2006, Chen *et al.*, 2007) because of their imperfect sensitivity and specificity and other associated limitations when applied at the individual animal level. An additional problem with the NSP tests is that they do not distinguish between infected animals that have eliminated virus (sterilising immunity) and those that are carriers (Kitching *et al.*, 2007).

(b) Salivary IgA ELISA

The diagnostic potential of FMDV-specific IgA antibodies, present in the mucosal secretions (saliva) of the oropharyngeal fluid, as an indicator of persistent infection (carriers) has been reported in the literature (Salt *et al.*, 1996, Moonen *et al.*, 2004a). Recently, a serotype-specific ELISA was developed to detect FMDV specific IgA antibody in the saliva of cattle (Parida *et al.*, 2006). The salivary IgA test was found to be useful in detecting sub-clinical infection in vaccinated cattle and therefore has potential for identification of persistently infected cattle irrespective of their vaccination statuses. However, this test is serotype specific and requires further validation before adoption for routine use in the field (Parida *et al.*, 2006).

2.7 Control and Eradication

The kind of control measures implemented in a country, region or district is dependent on the disease situation, the resources available, and the economic importance of the disease. In general, prevention and control strategies adopted can be categorised as those relating to a country where the disease is endemic and those in a country where the disease is usually absent. In any case, control activities could include a combination of vaccination, movement control of livestock and livestock products, stamping-out, zoosanitary measures and public awareness programmes.

2.7.1 Vaccines

The current FMD vaccine is produced from a suspension of the whole virus, inactivated with aziridine, and adjuvanted with oil or aluminium hydroxide. The VP1 polypeptide region of the virus is known to be immunogenic and is the major protective antigen (Davies, 2002). The FMD vaccine can contain one or more serotypes depending on the serotypes prevalent in a country. However, it is important that the vaccine contain the virus strains that match the field strains which are currently causing disease outbreaks. The FMD vaccine, like many other vaccines, does not induce sterilizing immunity and may therefore allow replication of virus when vaccinated animals are exposed to infection (Doel, 2003) thus giving rise to sub-clinical carriers. Aqueous based vaccines are administered to cattle, sheep and goats by the subcutaneous route, whereas the oil based vaccines are administered to cattle and pigs by the intramuscular route (Doel, 2003).

For countries where vaccination is routinely practiced, it is essential to differentiate between FMD infected and vaccinated animals for effective disease surveillance and control. Serological tests that detect antibodies to NSPs are currently used to do this. However, the current FMD vaccines, depending on their source, may contain traces of

NSP thus reducing the specificity of the NSP assays (Mackay *et al.*, 1998, Fowler *et al.*, 2008). Therefore, researchers are now more focused on the development of marker vaccines that can help differentiate immunity induced by vaccination from infection (Fowler *et al.*, 2008).

It is generally agreed for FMD control that at least 80% of the animals in a herd should be vaccinated in order to achieve protective herd immunity (Lombard and Shermbrucker, 1994). Protection of the herd as a whole reduces the opportunity for virus to enter, replicate and infect individual animals which do not have sufficient protective immunity (Doel, 2003). The current FMD vaccines are available as mono-, bi-, tri-, and tetravalent depending on the number of serotypes incorporated in the vaccine. Infection with one FMDV serotype does not induce protection against another serotype and therefore, essentially, infection with different serotypes can be considered as separate diseases. Because of these characteristics, control of FMD is a great challenge throughout the world.

2.7.2 Control measures in an endemic country or region

In countries or regions where FMD is endemic, the control measures usually employed include prophylactic vaccination using vaccines containing strains of FMDV prevalent in the area. The timing and frequency of vaccination will depend on the disease's epidemiology in the area. Strategic ring vaccination (during outbreaks) around the foci of infection is a measure to control the spread of FMD from the foci to other areas. Regulating and restricting movement of livestock and livestock products during an outbreak is important to prevent the spread of disease from infected areas to non-infected areas. It is also important to disinfect the premises in order to reduce the amount of virus in the environment so as to prevent the spread of disease through indirect means. These

control measures need to complement each other if there is to be any success in the control of the disease (Gibbens and Wilesmith, 2002, Wee *et al.*, 2008).

2.7.3 Control measures in an FMD-free country or region

In countries or regions desiring to retain disease-free status following an outbreak, stamping-out or culling of infected animals is usually done. This involves slaughter and disposal of infected animals followed by application of zoo-sanitary measures in the premises including disinfection of livestock markets, buildings, vehicles (Kitching, 1992, Sugiura *et al.*, 2001, Bates *et al.*, 2003). This method is usually used in developed countries where the government can afford to pay compensation to the farmers for the animals slaughtered. Culling has been successfully used for control and eradication of FMD in the UK during the 2001 FMD-epidemic (Anderson, 2002, Kitching *et al.*, 2006), during the 2000 outbreak in Japan (Sugiura *et al.*, 2001) and the 2002 outbreak in the Republic of Korea (Wee *et al.*, 2004). However, the success of this method will depend on the rapidity with which the infected animals or premises are detected. The longer the time interval between infection and implementation of culling, the longer it takes for disease to be contained. The success of this method also depends on effective implementation of other control measures such as movement control of animals and animal products during the stamping-out period (Gibbens and Wilesmith, 2002).

Regulating and restricting the movement of livestock and livestock products is crucial in countries where stamping-out is undertaken for control and eradication of FMD. For instance, the successful eradication of FMD in the UK (2001), Japan (2000) and Korea (2002) was due to effective implementation of movement restrictions along with culling.

In some FMD-free countries, vaccination is used along with culling to reduce the spread of disease during the period of culling. For instance, during the 2001 outbreak of FMD in the

Netherlands, unaffected animals in outbreak herds and in-contact herds around outbreak herds were initially vaccinated and subsequently all vaccinated animals were culled (Sutmoller *et al.*, 2003). However, due to socio-economic, political, animal and human welfare and ethical issues, stamping-out cannot be undertaken in countries where FMD is endemic or in underdeveloped countries.

2.7.4 Progressive control and Zoning

Given the infectious and transboundary nature of FMD, it would be difficult for countries to control the disease in isolation (FAO-OIE, 2004, Rweyemamu *et al.*, 2008b). Therefore approaches are adopted using the concepts of progressive control and zoning to effectively control FMD (Fujita, 2004, Anonymous, 2007e, Rweyemamu *et al.*, 2008a). Countries need to assess and define their national FMD status through sound epidemiological understanding of the virus maintenance and transmission so as to initiate this approach (Rweyemamu *et al.*, 2008a). Examples of regionally coordinated FMD control programmes based on progressive zoning approaches include the South-East Asia and China Foot-and-mouth Disease control and eradication campaign (SEACFMD) (Edwards, 2004, Edwards and Abila, 2004) and the Hemispheric FMD eradication plan for the Americas (PHEFA) (Correa Melo *et al.*, 2002). The SEACFMD goal is to eradicate FMD from SEA by 2020 (Anonymous, 2008b) whereas the PHEFA, which began in 1987, intended to control and eradicate FMD from the South American continent by 2009 (Correa Melo *et al.*, 2002). In South America, control programmes have been based on eco-systems and epidemiological information on the dynamics of FMD, the farming systems and cattle movement patterns to identify primary endemic areas (i.e. virus maintenance areas), secondary endemic areas (i.e. areas of virus propagation) and epidemic

areas (i.e. areas of explosive outbreaks) (Astudillo and Dora, 1987). The most cost-effective and sustainable strategy for FMD control was that which first targeted the primary endemic eco-system (Rweyemamu and Astudillo, 2002).

2.7.5 Foot-and-mouth disease Control Programme in Bhutan

In Bhutan, prior to the year 1996, methods to control FMD included barrier or ring vaccination, movement control and imposition of a ban on the importation of livestock and livestock products (Anonymous, 1996). This was, however, not done in an organized manner since there was no policy document and coordinating body to give directions to the control programme. The National FMD Control Programme, launched in 1996, set the stage for the organized and coordinated control of FMD through the publication of a policy document (Anonymous, 1996) and identification of a body to coordinate the control programme. The control programme was based on the disease's epidemiology and the country was divided into three zones: the epidemic zone which consisted of districts with frequent outbreaks and which were mainly located in the south of the country in areas bordering India; the endemic zone which consisted of districts with less frequent outbreaks; and the sporadic zone which contained districts with very few cases of FMD.

The current control programme is a revised version of the 1996-control policy document which came into effect in 2006 (Anonymous, 2005). The country is divided into three risk areas (high, medium or low) based on the disease's epidemiology.

2.7.5.1 Control in High Risk Areas

The high risk areas consist of districts bordering India where the disease incidence is much higher than in other districts. Bi-annual vaccination of all susceptible animals is being undertaken in the high risk areas with a trivalent vaccine (serotypes O, A and Asia 1)

covering at least 80% of the total susceptible population. All cloven-hoofed domestic animals, including small ruminants, are being vaccinated in this zone.

2.7.5.2 Control in Medium Risk Areas

In the medium risk areas, which include districts located in the interior parts of the country, bi-annual vaccination is done for two years following which the status is reviewed. Animals residing on either side of the highways are also vaccinated in this zone. Here too, a minimum of 80% vaccination coverage is maintained however only the large ruminants are vaccinated.

2.7.5.3 Control in Low Risk Areas

Districts which had sporadic outbreaks or no outbreaks are considered as low risk areas and vaccination is undertaken annually. Here too, a minimum of 80% vaccination coverage is maintained in the vaccinated population and only large ruminants are vaccinated.

Other additional control strategies currently being implemented include:

- Movement control of livestock and livestock products during an outbreak
- Imposition of a ban on the slaughter of animals during outbreaks
- Strengthening of the disease information system
- Strengthening of the cold chain maintenance of FMD vaccines

2.8 Disease Surveillance

Animal health surveillance is “an essential tool to detect disease or infection, to monitor disease trends, to facilitate the control of disease or infection, to support claims for freedom from disease or infection, to provide data for use in risk analysis, for animal and/or public health purposes, and to substantiate the rationale for sanitary measures” (OIE, 2009b). In general, surveillance can be divided into passive and active depending on the means by which the data are collected (OIE, 2009b). According to the OIE (2009b),

surveillance activities can also be classified into either structured population-based surveys or structured non-random surveillance. The former consists of surveillance done through systematic sampling at slaughter, randomised surveys, and surveys for infection in clinically normal animals including wildlife, while the latter consists of surveillance activities conducted through disease reporting, disease control programmes, ante-mortem and post-mortem inspections, laboratory investigation methods, sentinel herds, field observations and farm production records. In either case, surveillance data should be supported by other related information such as data on animal movements including transhumance; trading patterns of animal and animal products; epidemiology of the disease including environment, host and population distribution; and history of the importation and introduction of potentially infected animals (OIE, 2009b).

2.8.1 Structured population-based surveys

Sampling in structured population-based surveys can be done in two ways: non-probability based sampling methods such as convenience sampling or using expert opinion and probability based sampling methods such as randomised sampling (OIE, 2009b). The design of a structured survey is largely dependent upon the size and structure of the population being studied, the epidemiology of the infection and the resources available. Sampling should be done in such a way that the sample will be representative of the population, within the practical constraints imposed by different environments and production systems. The method used to calculate sample size for surveys depends on the purpose of the survey, the expected prevalence, the level of confidence desired of the survey results and the performance of the tests used (OIE, 2009b).

2.8.2 Surveillance to demonstrate freedom from infection

To confirm freedom from FMD, a surveillance programme targeting all susceptible species is required in a country/zone/region (OIE, 2009a). Either appropriately designed randomised sampling techniques or targeted surveillance of high risk species/locations/practices is undertaken to confirm disease freedom (OIE, 2009a). Surveillance to demonstrate freedom from infection should encompass three components: clinical, serological and virological surveillance. The main aim of clinical surveillance is to detect clinical signs of FMD through close physical examination of FMD-suspected animals. This can be done by direct examination of animals for clinical signs or indirectly through interviewing livestock owners/traders/workers (Bronsvort *et al.*, 2003). Suspected cases should then be confirmed by laboratory testing (OIE, 2009a). Serological surveillance is designed to detect antibodies against FMDV and appropriate tests must be used to differentiate natural infection from vaccine reactions or non-specific (false positive) reactions (OIE, 2009a). The serological surveys should be designed appropriately to give statistically valid results that can be extrapolated to the target population. Virological surveillance is used to monitor at-risk populations, confirm clinically suspected cases and to follow up on positive serological results (OIE, 2009a).

A country, which is free from FMD without vaccination, may choose serosurveillance of high-risk subpopulations and can use SP tests (OIE, 2009a). In contrast in areas where animals have been vaccinated, although SP tests can be used to monitor the serological response to the vaccination, NSP tests should be used to monitor for infection. Any positive herd should be resampled and tested with tests of high diagnostic specificity to reduce the number of false positives. If a positive result arises and vaccination is not practiced then the OIE (2009b) recommends confirmation using clinical, epidemiological, serological or virological methods in the positive and in-contact animals. Where a positive

result arises in an area where vaccination is practiced then it is recommended that all positive animals are retested after an adequate interval of time has lapsed and the NSP titres obtained at the second sampling compared with those initially found. Serum samples should also be collected and tested from animals that were in physical contact with the primary affected animals or from epidemiologically linked herds/flocks. The use of sentinel animals (young unvaccinated animals) is also recommended.

2.8.3 Participatory epidemiology

Participatory epidemiology (PE) is a relatively new branch of veterinary epidemiology which uses the concepts of participatory rural appraisal and rapid rural appraisal for collection of information on diseases based on the knowledge of livestock keepers (Catley, 2006). At the heart of this concept are the observations and knowledge of livestock keepers who have very detailed knowledge about their animals (Jost *et al.*, 2007). Common techniques used in PE include informal interviewing (questionnaires), visualisation and scoring methods, and focus group discussion. Participatory epidemiology has been used to understand disease epidemiology especially in marginalised areas (Thrusfield, 2005). Questionnaire interviews, which are one of the tools of PE, have been used in many FMD-endemic countries to understand the epidemiology of FMD (Cleland *et al.*, 1995a, Bronsvort *et al.*, 2003, Admassu and Ababa, 2006, Lindholm *et al.*, 2007, Al-Majali *et al.*, 2008) and other livestock diseases such as brucellosis (Gebretsadik *et al.*, 2007, Muma *et al.*, 2007, Solorio-Rivera *et al.*, 2007) mycoplasmosis (Gebretsadik *et al.*, 2007) and Johne's disease (Dhand *et al.*, 2007) based on the knowledge of the livestock keepers. Participatory epidemiology can be a cost effective method for epidemiological investigations of livestock diseases, especially in situations or areas constrained due to logistics and funds. However, data obtained through this method is subject to potential

biases because of likely occurrence of interviewer bias or interviewee bias and lack of statistical validity.

When investigating the epidemiology of any disease, a frequent start is examining existing data on the disease of interest. In the following chapter historical data on FMD in Bhutan is collected and analysed to further understand the epidemiology of this disease in the country.

Retrospective study of the epidemiology of FMD in Bhutan

This Chapter has been published in a peer-reviewed journal:

Dukpa, K., Robertson, I.D., Edwards, J. and Ellis, T.M. (2011) A retrospective study on the epidemiology of foot-and-mouth disease in Bhutan. *Tropical Animal Health and Production*, **43** (2), 495-502.

3.1 Introduction

The success of any disease control programme depends on a clear understanding of the epidemiology of the disease (Perry *et al.*, 2002). The first step in the control of any disease involves the analysis of existing data to understand the distribution and patterns of spread of the disease (Ayebazibwe *et al.*, 2010). Epidemiological analysis of retrospective data collected through passive surveillance can yield important information about the possible source and nature of a disease (Bhattacharya *et al.*, 2005, Khounsy *et al.*, 2008, Verma *et al.*, 2008). The epidemiology of FMD in Bhutan in terms of spatiotemporal distribution, species susceptibility, patterns of occurrence and risk factors has not been previously investigated. Although outbreak data collected through passive surveillance is available in Bhutan, to date, no analysis has been undertaken of these data to further our understanding of the disease's epidemiology.

Therefore, this study was undertaken with the following objectives:

- To describe the spatiotemporal distribution of reported outbreaks of FMD in Bhutan during the period 1996 to 2008
- To generate baseline epidemiological data on FMD in Bhutan

3.2 Materials and methods

3.2.1 Data

Data on outbreaks of FMD between the years 1996 and 2008 were obtained from the VIS maintained by the NCAH. The disease was diagnosed through the country's passive surveillance system operated by a network of veterinary centres and laboratories located strategically across the kingdom (Chapter 1). The serotyping data received from the WRLFMD for the outbreaks of FMD from 1982 until 2008 were also included in the analysis reported in this chapter.

For the purpose of this chapter, a *case* is defined as an animal with clinical signs or lesions characteristic of FMD with or without laboratory confirmatory diagnosis. An *outbreak* is defined as the occurrence of one or more *cases* of FMD in a herd, village or sub-district. A *case* is considered as a separate *outbreak* if it occurs in a herd or village separated from other herds or villages by physical barriers such as rivers, streams, hills or mountains. *Cases* occurring at the same time in villages or herds that were contiguous were considered as one *outbreak*.

Since the exact location of the affected herds was not recorded, the geographical location of the *cases* was approximated using the point location of the villages and centroids of the sub-districts in which the *cases* were reported. A software mapping programme (ArcMap 9.3, ArcGIS, ESRI, Redlands, CA) was used to map the outbreaks for spatial analysis.

3.2.2 Statistical analysis

Descriptive analysis was conducted in Excel (Microsoft® Office Excel 2003) and statistical tests were undertaken in SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Data were checked for normality by performing the normality function tests such as the Kolmogorov-Smirnov and Shapiro-Wilk tests (Pallant, 2005). ANOVA (parametric tests) was used for normally distributed data. For data that were not normally distributed, non-parametric tests including the Chi-square and Mann-Whitney U tests were performed to check for any statistical differences in the incidence of outbreaks in different groups. Spearman's rank order correlation was used to investigate the correlation between the livestock population and the incidence of disease.

3.3 Results

A total of 230 outbreaks of FMD were recorded at the sub-district level from the 1 January 1996 to 31 December 2008 (average of 18 outbreaks per year). A total of 11,491 cattle, 211 pigs, 41 goats and 56 sheep were recorded to have been affected with FMD during this 13-year period.

3.3.1 Spatial distribution

The disease was reported in 299 villages from 19 of the 20 districts during this period. No occurrences of disease were reported in the district of Gasa during this period. Chukha (n=57, 24.8%) had the highest number of outbreaks followed by Sarpang (n=29, 12.6%), and Thimphu (n=17, 7.4%). Tsirang (n=2, 0.9%) had the lowest number of outbreaks (Figure 3.1) and has not reported outbreaks of FMD since 1998 (Anonymous, 2009b). Approximately 50% (106/205) of the sub-districts (*geogs*) in the country had a reported occurrence of one or more outbreaks of FMD during this period.

The distribution of outbreaks per 1000 cattle population at the district level and per 100 cattle at the sub-district level, based on the 2007 livestock census data (Anonymous, 2009a), is displayed in Figures 3.2 and 3.3, respectively.

Figure 3.1 Reported numbers of outbreaks of FMD at the district-level in Bhutan (1996-2008)

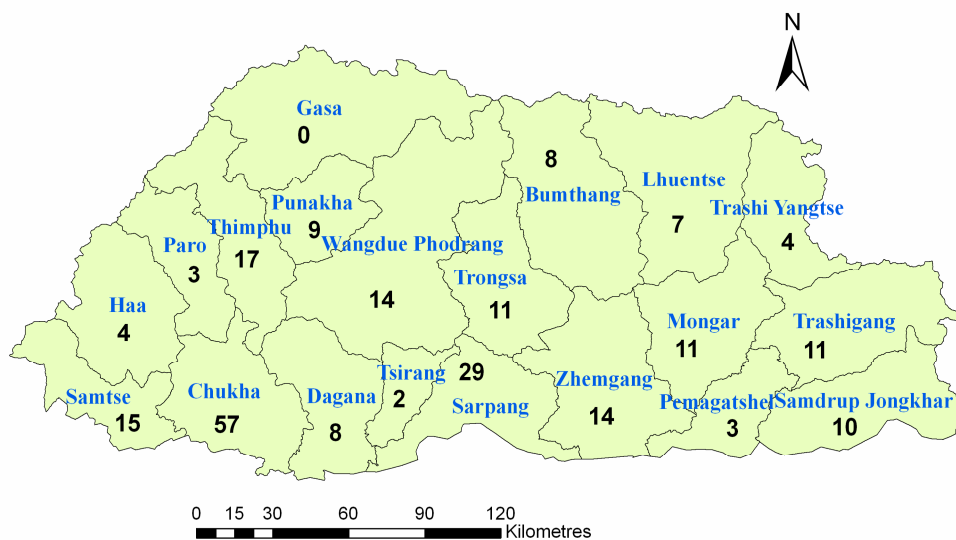


Figure 3.2 Choropleth map of incidence of outbreaks of FMD per 1000 cattle at the district level (1996-2008)

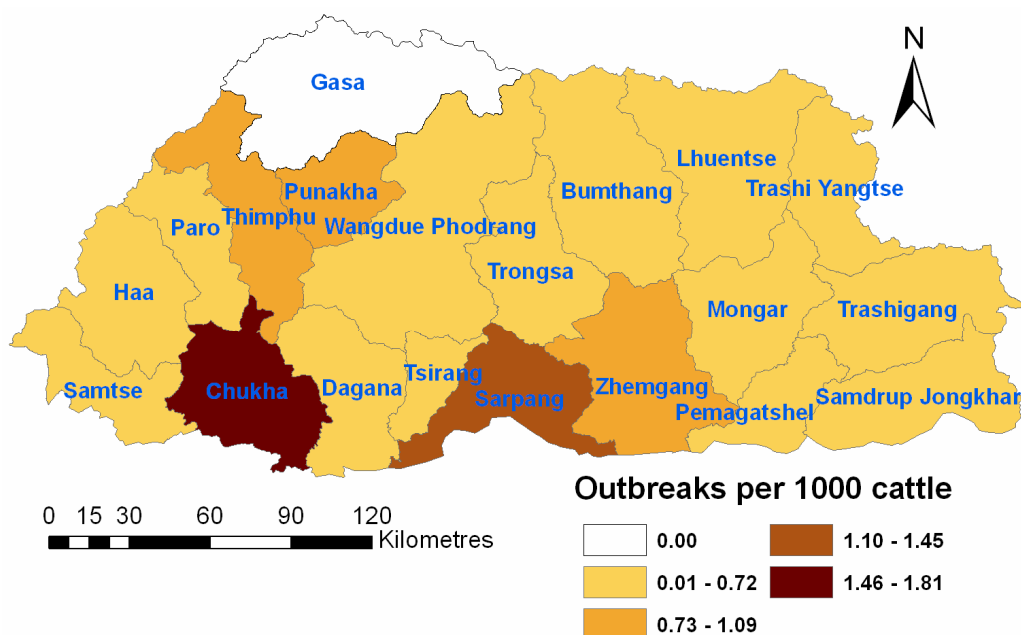
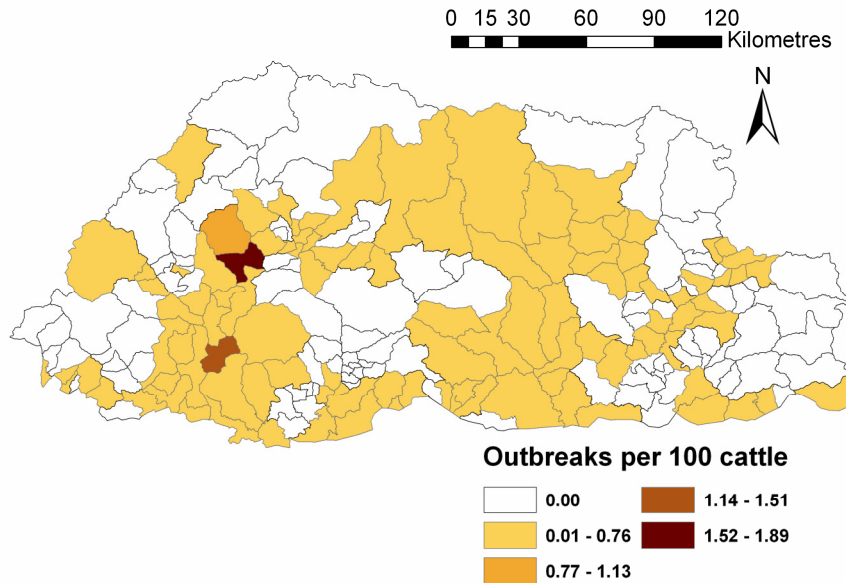


Figure 3.3 Choropleth map of incidence of outbreaks of FMD per 100 cattle in the sub-districts of Bhutan (1996-2008)

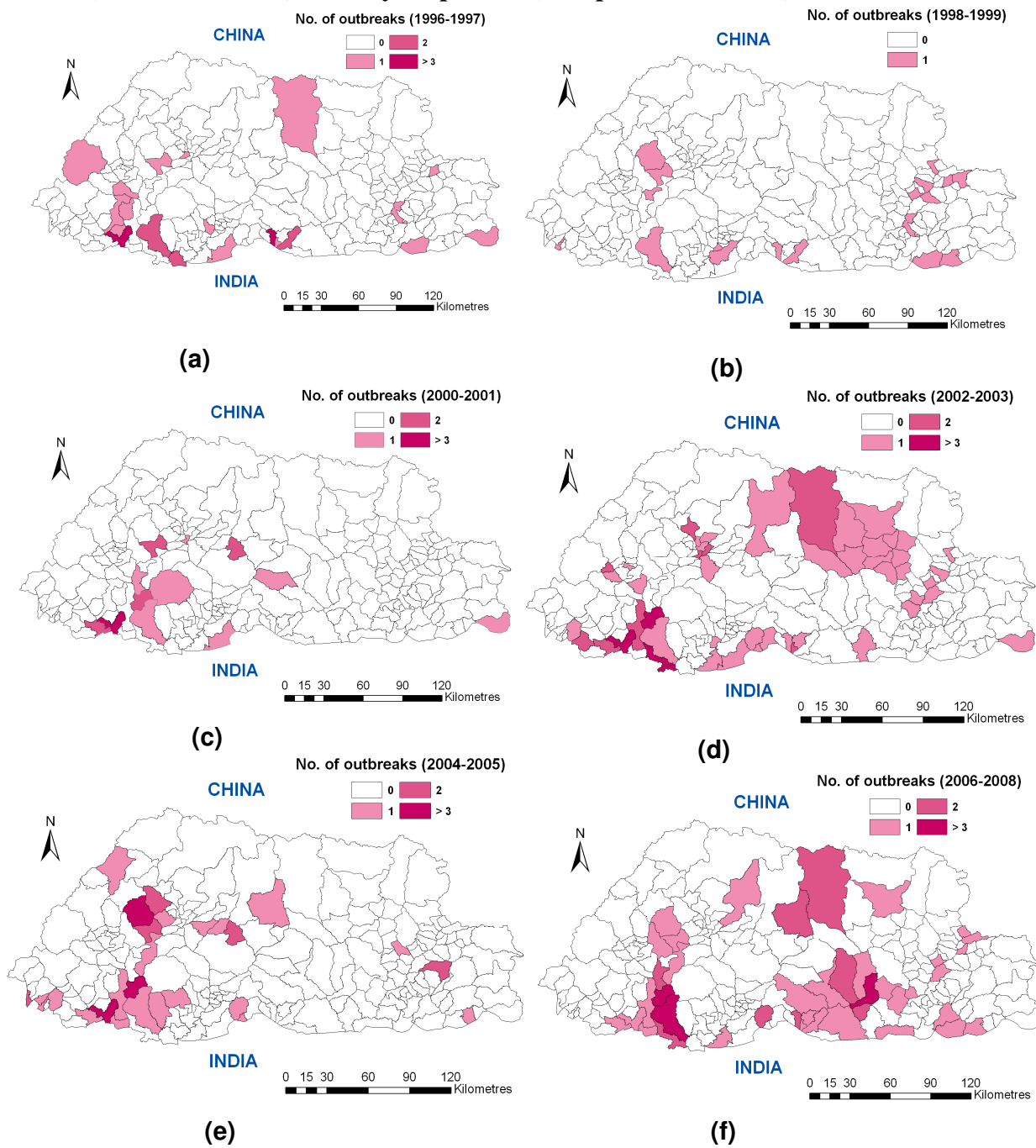


Chukha had the highest incidence over the 13-year period of 1.81 outbreaks per 1,000 cattle followed by Sarpang (1.39) and Thimphu (1.05) (Figure 3.2). Chang sub-district (Thimphu District) had the highest outbreak incidence of 1.89 outbreaks per 100 cattle during the study period (Figure 3.3) followed by Bjachho (Chukha District) (1.21) and Kawang (Thimphu district) (0.89). The year-wise spatial pattern of the incidence of FMD during the study period in Bhutan is displayed in Figure 3.4 (a-f).

3.3.2 Temporal distribution

A high annual incidence of FMD was reported in 2002 (34 outbreaks), 2003 (n=31) and 2007 (n=34) (Figure 3.5). There were no significant differences between the years on the annual incidence of FMD ($P=0.998$, $t=-0.003$, $df=12$). The highest monthly incidence was reported in the month of August (n=36) which accounted for 15.7% of all outbreaks (Figure 3.6). The lowest incidence was in November (n=10, 4.3%). There was no significant difference in the incidence of outbreaks between the months ($P=0.989$, $t=-0.014$, $df=11$).

Figure 3.4 Choropleth maps showing the spatial distribution of outbreaks of FMD in Bhutan (sub-district level) over 2-year periods (Except for 2006-2008)



Note: (a) Jan 1996--Dec 1997, (b) Jan 1998--Dec 1999, (c) Jan 2000--Dec 2001, (d) Jan 2002--Dec 2003, (e) Jan 2004--Dec 2005, (f) Jan 2006--Dec 2008

Figure 3.5 Annual distribution of outbreaks of FMD in Bhutan

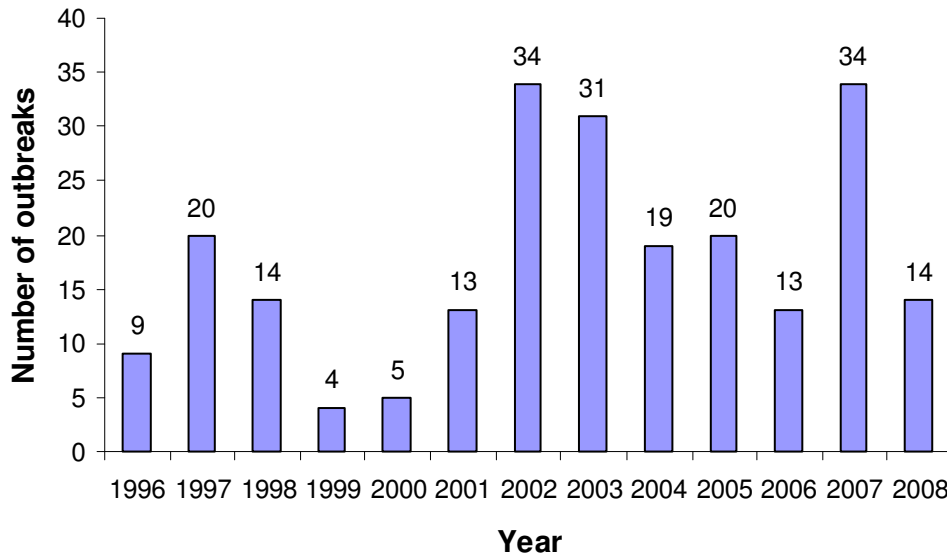
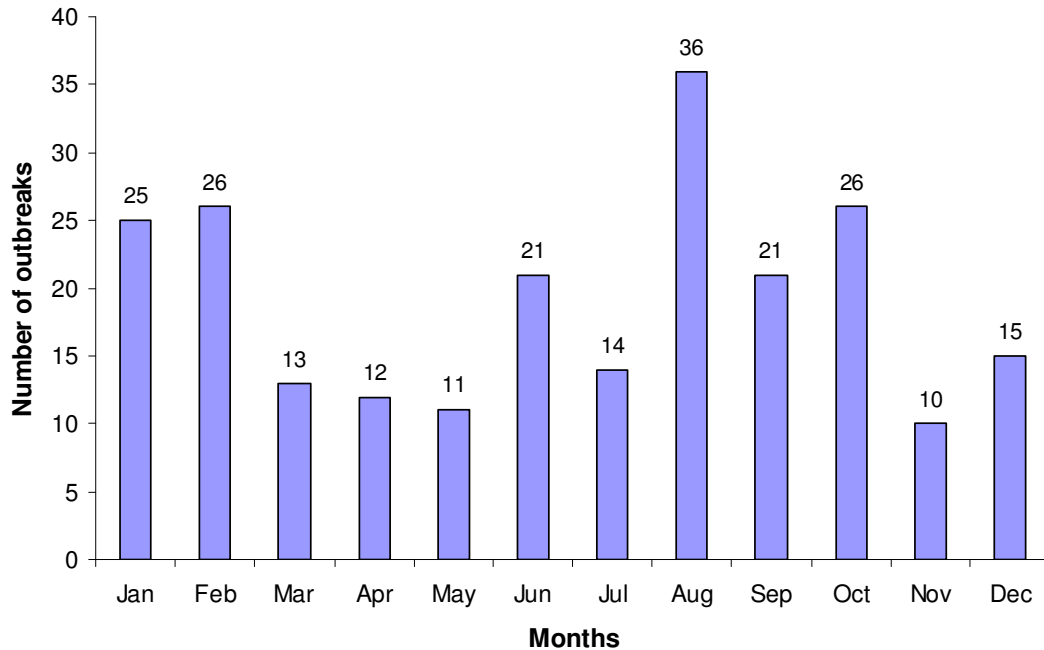
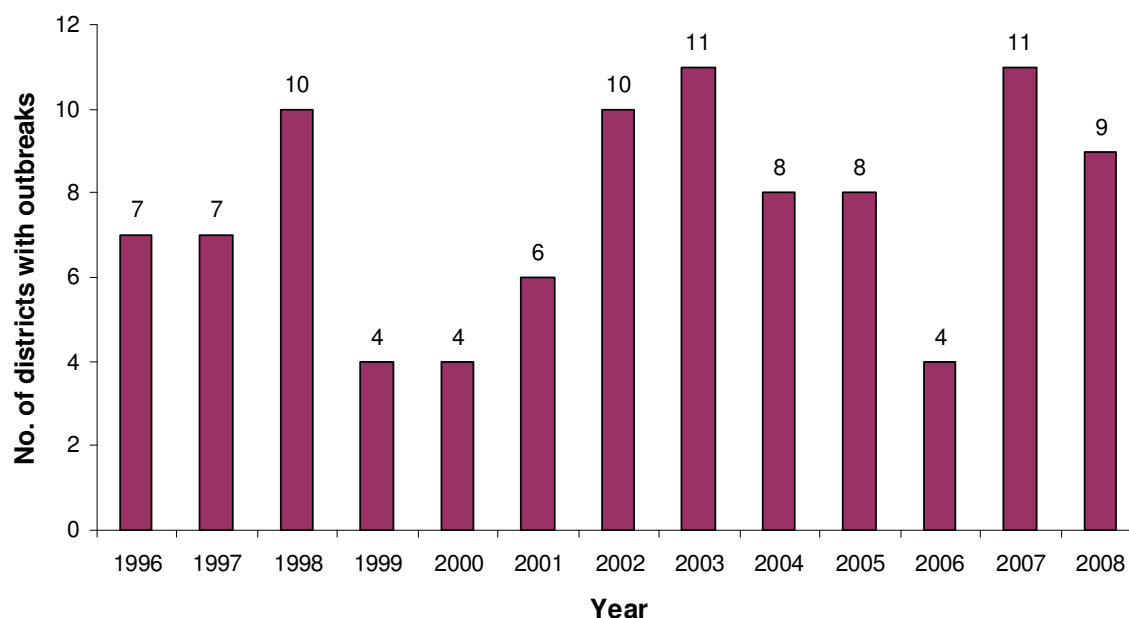


Figure 3.6 Monthly distribution of outbreaks of FMD in Bhutan (1996-2008)



On average 7.5 districts (± 2.5 SD) had outbreaks of FMD every year. The highest number of districts with outbreaks were in 1998 and 2002 ($n=10$), and in 2003 and 2007 ($n=11$) (Figure 3.7). However, there was no significant difference in the number of districts reporting FMD over the 13-year period ($P=0.982$, $t=0.023$, $df=11$).

Figure 3.7 Number of districts recording an outbreak of FMD each year during the period 1996 to 2008



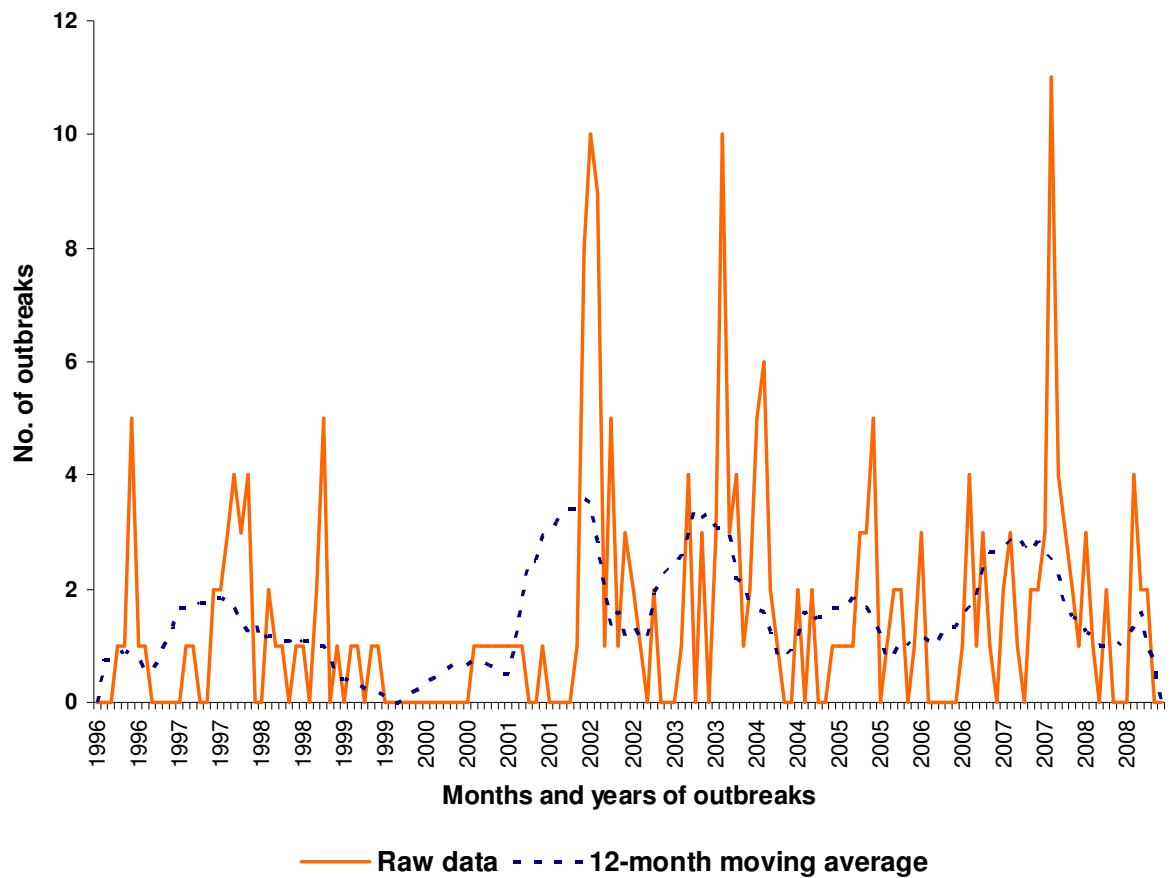
When the overall data were grouped into four seasons, more outbreaks were reported in summer (Jun-Aug, n=71, 30.9%) followed by winter (Dec-Feb, n=66, 28.7%) and autumn (Sep-Nov, n=56, 24.3%). The lowest number was in spring (Mar-May, n=37, 16.1%). There was a significant difference in the number of outbreaks reported between seasons ($P=0.008$, $\chi^2 = 11.77$, $df = 3$).

In order to check whether the location of the sub-districts had a confounding effect on seasonality, the data were dichotomized into two categories: sub-districts located in the south (n=28), below 1,000 m.a.s.l., which experienced hot summers and cool winters, and those located in the north (n=78), above 1,000 m.a.s.l., which experienced comparatively cooler summers and cold winters. The sub-districts in the south had more outbreaks in summer (n=32), followed by autumn (n=23), winter (n=21) and spring (n=18). However these differences were not statistically significant ($P=0.2$, $\chi^2=4.63$, $df=3$). The sub-districts in the north had, however, significant ($P=0.008$, $\chi^2=11.82$, $df=3$) seasonal differences with

more outbreaks reported in winter (n=45) followed by summer (n=39), autumn (n=34) and spring (18).

The time series distribution of outbreaks of FMD, by month and year, for the entire 13-years is displayed in Figure 3.8. A 12-month moving average curve was plotted to visualize the temporal pattern of FMD over the 13-year period. There were peaks of outbreaks in the years 1997/1998, 2002/2003, and 2007/2008 during which the PanAsia strain of the O serotype was involved.

Figure 3.8 Time series distribution of outbreaks of FMD in Bhutan



3.3.3 Species affected

Cattle were the most predominant species affected, being involved in all of the outbreaks reported. Pigs were the second most commonly affected domestic species being involved in 18 outbreaks (7.8%) followed by goats (n=13, 5.7%) and sheep (n=3, 1.3%). Thirty-two

outbreaks had two species involved; 11 outbreaks had three species; and two outbreaks had all four species involved. Only one species (cattle) was involved in 254 outbreaks.

3.3.4 Serotypes

The outbreaks from 1982 to 2008 were caused by serotypes O, A, Asia 1 and C (Table 3.1). The last recorded outbreaks of FMD in Bhutan due to serotypes C, Asia 1 and A were in the years 1991, 2002 and 2003, respectively.

(http://www.wrlfmd.org/fmd_genotyping/asia/bhu.htm accessed on 8 September 2009).

Serotype O, which constituted 70.6% of the outbreaks typed, was the most predominant serotype followed by serotypes A (16.7%), Asia 1 (8.8%) and C (3.9%). Serotype O has been the only serotype detected in Bhutan for the last six years. The O serotype isolated in Bhutan between 1998 and 2007 belonged to the Middle East-South Asia (ME-SA) toptotype. The spatial distribution of the serotypes isolated between 1982 and 2008 is summarized in Figure 3.9.

The district of Chukha was the only district that had outbreaks caused by all four serotypes (O, A, C, and Asia 1) during the study period. Three serotypes were reported in Dagana (O, A and Asia 1) and two in Paro (C and Asia 1). Serotypes A and O were detected in outbreaks from Haa, Thimphu, Wangdue Phodrang and Lhuentse. In all other districts only serotype O was isolated from the samples submitted to the WRLFMD. Serotyping results for outbreaks from Tsirang, Pema Gatshel, and Trongsa were not available due to the failure to submit samples to the WRLFMD.

Table 3.1 Distribution of FMDV serotypes in Bhutan (1982 to 2008)

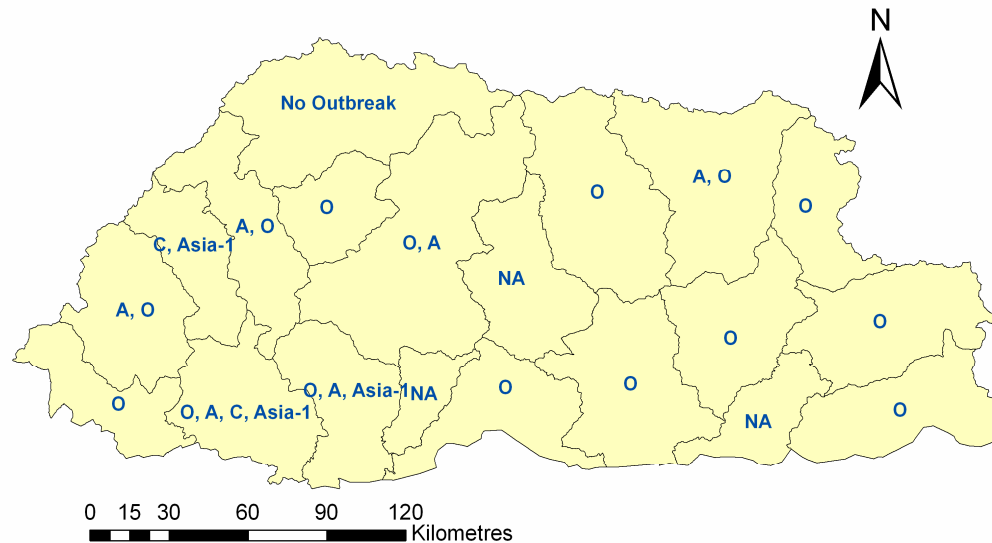
Year	No. of outbreaks	Serotype	WRLFMD validated?	Species affected	Districts
1982	NA	A	Yes	Cattle	^a Thimphu
1983	NA	O	Yes	Cattle	^a Thimphu
1984	NA	A	Yes	Cattle	^a Thimphu
1985	NA	O	Yes	Cattle Pigs	^a Thimphu
1986	NA	Asia 1	Yes	Cattle	^a Dagana
1990	NA	A	Yes	Cattle	^a Haa
1990	NA	O	Yes	Cattle	^a Samtse
1991	NA	C	Yes	Cattle	^a Paro, ^a Chukha
1993	NA	O	Yes	Cattle	^a Samdrup Jongkhar
1994	NA	Asia 1	Yes	Cattle	^a Thimphu
1996	9	O, A	Yes	Cattle	^a Trashigang ^b , ^a Sarpang
^c 1997	20	NA	No	Cattle, Pigs	Chukha, Dagana, Haa, Pemagatshel, Punakha, Samdrup Jongkhar, Sarpang
1998	14	O	Yes	Cattle Pigs, Goats	^a Thimphu, ^a Sarpang, ^a Trashigang, Haa, ^a Mongar
^c 1999	4	NA	No	Cattle Goats	Samdrup Jongkhar, Sarpang Thimphu, Trashigang
^c 2000	5	NA	No	Cattle	Chukha, Samdrup Jongkhar Samtse, Sarpang
2001	13	O	Yes	Cattle Pigs, Goats	^a Chukha, Dagana, Samtse Thimphu, Trongsa, Wangdue Phodrang

Table 3.1 (continued) Distribution of FMDV serotypes in Bhutan (1982 to 2008)

Year	No. of outbreaks	Serotype	WRLFMD validated?	Species affected	Districts
2002	34	O, A, Asia 1	Yes	Cattle Yaks, Pigs	^a Lhuentse ^b , ^a Punakha ^a Chukha ^d , ^a Mongar ^a Bumthang, ^a Paro ^d
2003	31	O, A	Yes	Cattle, Sheep Pig, Goats,	^a Sarpang, ^a Wangdue Phodrang ^b Trashigang, ^a Mongar ^a Punakha, ^a Chukha ^b
2004	19	O	Yes	Cattle	^a Chukha, Thimphu
^c 2005	20	NA	No	Cattle Pigs, Goats	Chukha, Dagana, Punakha Samdrup Jongkhar, Samtse Thimphu, Trongsa, Wangdue Phodrang
^c 2006	13	NA	No	Cattle Pigs	Chukha, Samdrup Jongkhar Thimphu, Trongsa
2007	34	O	Yes	Cattle, Goats Sheep, Pigs	^a Sarpang, ^a Zhemgang
2008	14	O	Yes	Cattle, Pigs	^a Chukha, Zhemgang Thimphu, Mongar

NA Not available; ^a Serotyping done; ^b A serotype; ^c years in which no samples were submitted to WRLFMD; ^dAsia 1,

Figure 3.9 Distribution of serotypes of FMDV in Bhutan (1982 to 2008)



Note: NA means information not available

3.3.5 Risk factors for outbreaks of FMD in Bhutan

There was no significant difference in the median number of outbreaks between districts sharing a border with India and those that didn't ($P=0.370$, Mann-Whitney U test). Districts bordering India reported a total of 136 outbreaks compared with 94 in those districts not sharing a border with India. Those sub-districts sharing a border with India had significantly more outbreaks than those that didn't ($P=0.001$, Mann-Whitney U test, $z=-3.411$).

Using the cattle population from the 2007 census (Anonymous, 2009a) and the existing outbreak records, a significant positive correlation was found between the cattle population and the incidence of disease at the district level ($r^2=0.505$, $P=0.023$, Spearman's rank order correlation). When the districts were grouped into four regions (South=Samtse, Chukha, Sarpang, Zhemgang, Pema Gatshel, Dagana, Samdrup Jongkhar; West=Thimphu, Haa, Gasa, Paro, Punakha; Central =Wangdue Phodrang, Bumthang, Trongsa, Tsirang; East=Trashigang, Trashi Yangtse, Mongar, Lhuentse), regions with a high cattle

population had significantly more outbreaks than did regions with low cattle population ($r^2=0.949$, $P=0.026$, Spearman's rank order correlation).

3.3.6 Major outbreaks of FMD in Bhutan

Temporal patterns of outbreaks indicate that there have been waves of outbreaks in Bhutan. The first wave started in 1997/1998 when 34 outbreaks were recorded over a 2-year period (Figure 2.9). Thereafter the next epidemic wave started in 2002/2003 when 65 outbreaks were reported. A total of 2694 cattle and six pigs were affected and 38 deaths in cattle were reported during the 2002/2003 epidemic. The last epidemic wave occurred in 2007/2008 when 48 outbreaks were recorded affecting 50% of the districts in the country. The 2007 epidemic affected 2857 cattle with 404 deaths within a period of 5 months. Each of these three epidemic episodes was as a result of the incursion of the PanAsia strain of the O serotype from neighbouring countries, possibly, through the transboundary movement of animals (Hemadri *et al.*, 2002, Knowles *et al.*, 2005).

Phylogenetic analysis of the samples submitted to the WRLFMD demonstrated that the PanAsia strain of the O serotype was involved in the outbreaks in 2002 and 2003 (Knowles *et al.*, 2005, Schumann *et al.*, 2008). Although serotype O was isolated from most of the outbreaks in 2002, serotypes A (Lhuentse district) and Asia 1 (Chukha and Paro) were also found to be involved along with the O serotype. The isolate (O/BHU/7/2002) from the 2002 outbreak was found to be closely related to an isolate (O/NEP/1/2003) from Nepal (Knowles *et al.*, 2004) and three isolates (O/IND/83, 96, 116/2001) from India (Knowles and Davies, 2002). The isolate (O/BHU/41/2002) was found to be closely related to two Indian isolates of 2000 (O/IND/16/2000) and 2001 (O/IND/24/2001) (Schumann *et al.*, 2008).

Similarly, most of the isolates from the 2003 outbreak (O/BHU/41, 14, 15/2003) were closely related to isolates from Nepal (O/NEP/2, 4, 5/2003) (Knowles *et al.*, 2004). The isolates from the 2007 outbreak (O/BHU/11, 12, 18/2007) were closely related to the Bhutanese isolates of 2004 and the isolates from India in 2001 (O/IND/136, 155, 156/2001) (Knowles and Wadsworth, 2007).

The next major outbreak, which occurred in an epidemic form in one of the remote districts, took place in 2007. A description of this outbreak and its detailed epidemiological features are covered in Chapter 4.

3.4 Discussion

This is the first retrospective analysis of outbreaks of FMD in Bhutan and covers outbreaks between 1996 and 2008. The disease occurred in high-endemic form in the districts bordering India and in a low-endemic form in the interior districts. Those sub-districts bordering India were found to have significantly more outbreaks than those not bordering India. Therefore, for future disease control, it would be appropriate to consider the disease situation at the sub-district level rather than at the district level. The high-risk sub-districts sharing a border with India should be targeted for a disease surveillance and control programme.

Spatial analysis demonstrated that FMD was persistently present in districts such as Chukha and Sarpang. These two districts together accounted for 37% of the total outbreaks. The reasons for this could be multiple. Firstly, these districts share long and porous borders with India and anecdotal evidence suggests that there is widespread mixing of animals from India and Bhutan at common grazing and watering areas. Foot-and-mouth disease is endemic in the neighbouring Indian States of West Bengal (Basu *et al.*, 1999, Bhattacharya *et al.*, 2005) and Assam (Sarma and Sutopa, 2003, Sanjoy and Sarma, 2005)

and therefore there is a high probability of the infection spreading across the border through the mixing of cattle. In order to understand the role of animal movements (legal and illegal) in the epidemiology of FMD in Bhutan, there is a need to undertake further studies to compare animal movement patterns between endemic and non-endemic districts so as to find out the factors responsible for differences in disease incidence. Such a study is reported in Chapter Eight wherein the animal movement patterns in Sarpang, an FMD-endemic district, and Tsirang, an FMD-free district, are compared.

The main highway in Bhutan connecting Phuentsholing, the commercial hub of Bhutan, with Thimphu, the capital city, runs through Chukha district. Chukha is also one of the most industrialized districts with two hydropower projects and newly established industries. Sarpang district is also the main commercial hub for central Bhutan. This would result in significant traffic, both by humans and animals, through these districts. Chukha had 10.2% of all FMD-susceptible animals in Bhutan in 2007 (Anonymous, 2009a). In this study livestock density was found to be positively correlated with disease incidence. Similar findings have been reported by others in countries with similar livestock management systems (Khounsy *et al.*, 2008, Verma *et al.*, 2008). In future control programmes, it is recommended to prioritise disease control activities to districts with high livestock populations to optimize the use of scarce resources.

Temporal analysis showed that outbreaks were reported every year, although the frequency of outbreaks varied from year to year. The disease occurred in epidemic proportions once every 4 to 5 years as was seen in 1997/1998, 2002/2003 and then in 2007/2008. The epidemic wave in 1997/1998 in Bhutan saw the appearance of a new strain, the PanAsia strain, of the O serotype. This pandemic strain could have entered Bhutan through the movement of livestock from across the border, since this strain was thought to have been present in India as early as 1982 (Hemadri *et al.*, 2002, Knowles *et al.*, 2005). The

pandemic PanAsia strain was again found to be involved in the 2002/2003 outbreaks and sequence analysis showed close relationship with the virus isolates circulating in India and Nepal. Since Bhutan imports cattle mainly from India and Nepal, it is likely that the PanAsia strain was again introduced into the country through the movement of animals. However since none of these animals were tested for FMD at the time of entry, there is no concrete evidence to confirm this theory. There is, therefore, a need to have an effective FMD surveillance programme, especially in the quarantine stations at the border areas. All imported animals need to be serologically screened against FMD before they are allowed to enter the country.

Cattle were the main species affected with FMD in Bhutan. Sheep, goats and pigs were rarely affected and do not appear to play a significant role in the epidemiology of FMD in Bhutan. During the study period, serotype O was the most dominant serotype in Bhutan, consistent with the disease epidemiology in India (Bhattacharya *et al.*, 2005, Sanjoy and Sarma, 2005) and Nepal. The other serotypes were rarely isolated from the outbreaks during the study period and therefore do not seem to play an important role in the epidemiology of FMD in Bhutan.

More outbreaks were reported in sub-districts in the south during summer while in northern sub-districts more were reported during winter. Summers, especially in the southern part of Bhutan, can be very hot and humid. Given the extensive system of livestock management the inclement weather could possibly trigger outbreaks of disease in stressed animals. Carrier cattle can transmit virus under undefined “trigger” conditions in the field (Alexandersen *et al.*, 2002). The other reason could be the increased movement of livestock during the months of May-July (summer) when cattle are used for preparation of rice fields. Stress can suppress the development of immunity and therefore can predispose

an animal to infection (Dohms and Metz, 1991). Climatic stress, due to a hot and humid environment, can also adversely affect livestock production (Somparn *et al.*, 2004).

Although FMD is endemic in Bhutan, no FMD was reported in Gasa district during this period. This could be due to the small cattle population which constituted only 0.2% of the total cattle population in Bhutan (Anonymous, 2009a), or due to the presence of natural barriers in terms of snowcapped mountains to the north and a lack of roads connecting with other districts. However, Tsirang district, although surrounded by FMD-endemic districts, has had no reported outbreaks of FMD for the past 11 years. Therefore, there is a need to validate this disease-free status in order to have confidence in the passive surveillance system of Bhutan. The animal movement patterns in these districts needs to be compared with that from endemic districts to determine the reasons for the differences in the disease incidence.

Because of the low operational cost and the availability of readily available data, passive surveillance can be of immense use for countries, such as Bhutan, where resources are limited to conduct active surveillance. Since FMD can be diagnosed at the field level based on clinical signs, especially in cattle, passive surveillance can play an important role in the overall disease surveillance system in Bhutan. As highlighted in Chapter One, Bhutan has the necessary veterinary infrastructure and staffing (veterinarians and para-veterinarians) to enable disease surveillance to be undertaken on a continuous basis. Retrospective studies, based on analysis of data collected through a passive surveillance system, have contributed greatly in understanding the epidemiology of FMD in other endemic countries such as Lao PDR (Khounsy *et al.*, 2008), India (Sarma and Sutopa, 2003, Bhattacharya *et al.*, 2005, Verma *et al.*, 2008), Nepal (Ferris *et al.*, 1992), Mali (Sangare *et al.*, 2004), and Paraguay (Peralta *et al.*, 1982).

However, the findings of this study need to be interpreted with caution because of the likely bias of underreporting of cases and outbreaks which is inherent in a passive surveillance system (McLeod, 2003, Thrusfield, 2005, Sumption *et al.*, 2008). The biases occurring from non-reporting of sub-clinical cases are expected to be low given the fact that cattle are the predominant species in Bhutan and the clinical signs are more discernible in this species compared to other FMD-susceptible species (Davies, 2002).

In conclusion, for the first time in Bhutan data on outbreaks of FMD, collected through years of passive surveillance, have been analysed and described. The study has confirmed that FMD is endemic to Bhutan and sub-districts bordering India are at higher risk of infection than those located in the interior parts of the country. The study reported the significance of the livestock population as a determinant for the occurrence of FMD. The study highlighted the incursion of new strains of FMDV, in particular the PanAsia strain of the O serotype, into Bhutan possibly through transboundary movement of animals and the need for active surveillance in the quarantine stations and border areas. The study also highlighted the significance of the O serotype and cattle as the main indicator species in the epidemiology of FMD in Bhutan. The findings from this study can be used as baseline epidemiological data for further research to understand the epidemiology of FMD in Bhutan.

The study has identified several critical factors to understanding the epidemiology of FMD in Bhutan which this thesis will address. There is a need to identify the risk factors for FMD in Bhutan in order to explain the variation in the disease incidence across different agro-ecological zones and different farming systems. There is also a need to undertake serological and clinical surveillance to validate and provide confidence in the passive surveillance system. There is also a need to understand animal movement patterns in the

country in order to explain the variation in disease incidence across different agro-ecological zones. These aspects will form the basis for the following chapters of this thesis.

The Epidemiological Characteristics of the 2007 FMD Epidemic in Sarpang and Zhemgang districts of Bhutan.

This Chapter has been published in a peer-reviewed journal:

Dukpa, K., Robertson, I.D. and Ellis, T.M. (2011) The Epidemiological Characteristics of the 2007 Foot-and-Mouth Disease Epidemic in Sarpang and Zhemgang Districts of Bhutan. *Transboundary and Emerging Diseases*, **58** (1), 53-62.

4.1 Introduction

In the previous chapter the distribution, patterns of occurrence and risk factors for FMD in Bhutan based on the existing data were discussed. Although, the PanAsia strain of the O serotype was involved in three waves of epidemic outbreaks in Bhutan, there is no information about the behaviour of this FMDV strain under different control strategies in Bhutan. Studies on epidemiological characteristics of outbreaks of FMD can provide valuable insights into the different aspects of the disease patterns and can be useful for planning future disease control programmes (Gibbens *et al.*, 2001, Moutou, 2002, Perry *et al.*, 2002, Gallego *et al.*, 2007). A thorough understanding of the epidemiology of FMD is essential for developing effective surveillance, control and eradication programmes (Al Khamis *et al.*, 2009).

Diseases are known to cluster at various levels and for different reasons, and therefore, it is essential to identify such clusters in order to understand more clearly the pattern of disease

transmission and ways to control the disease (Carpenter, 2001). Spatiotemporal techniques can be used to assess the presence of disease clusters, by time and space. Clusters of cases can be further assessed to identify predisposing factors for outbreaks or epidemics and to evaluate the effectiveness of existing control programmes (Ward and Carpenter, 2000, Carpenter, 2001).

The study outlined in this chapter was done with the following objectives:

- To compare the epidemiological characteristics of the 2007 FMD outbreak in two districts with different levels of disease prevention and control.
- To detect spatial and temporal clusters in order to understand the potential source and spread of the epidemic.

4.2 *Materials and Methods*

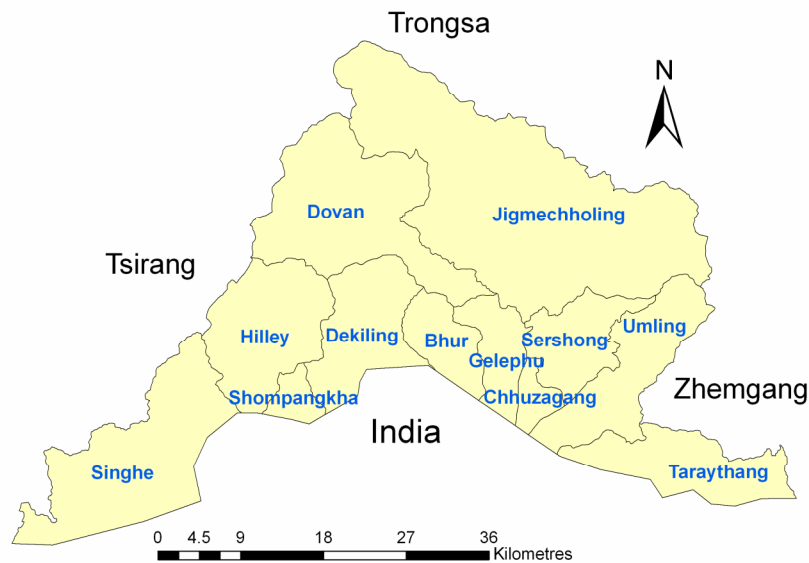
4.2.1 Study area

This study was undertaken in the districts of Sarpang and Zhemgang which were affected by the 2007 epidemic of FMD.

4.2.1.1 Sarpang

Sarpang (Figure 4.1) is located in the southern foothills of Bhutan and has altitudes ranging from 200 to 3800 m.a.s.l. (Anonymous, 2002c).

Figure 4.1 Map of Sarpang district with its 12 sub-districts



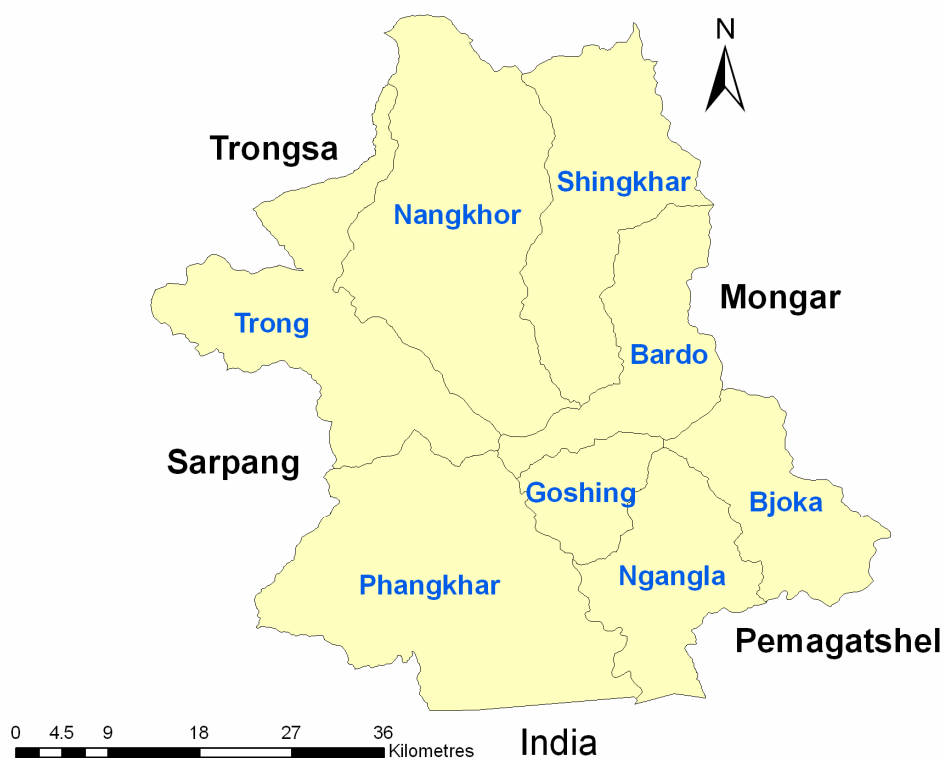
The district shares a border with the neighbouring Indian state of Assam, where FMD is endemic (Sarma *et al.*, 1983, 1985). The district experiences hot and humid summers with abundant rainfall from May to September and a cool dry season from October to February. Foot-and-mouth disease is endemic in Sarpang (Anonymous, 2009b). The last outbreak, before the study period, was recorded on 6th January 2004 in the Hilley sub-district (Dukpa, 2004). Since the launch of the revised NFMDCP in 2005, the district undertook bi-annual vaccination (against serotypes O, A and Asia 1) starting in July 2006. Cattle (n=20,583, 78.5%) are the most predominant among the FMD-susceptible species in Sarpang followed by goats (n=3,046, 11.6%), pigs (n=1,794, 6.8%), sheep (n=600, 2.3%) and buffalo (n=237, 0.9%) (Anonymous, 2009a).

4.2.1.2 Zhemgang

Zhemgang (Figure 4.2) is located in the south-central part of the country and there have only been sporadic outbreaks of FMD reported in this district (Dukpa and Tenzin, 2007, Anonymous, 2009b). Zhemgang is one of the most inaccessible districts in the country with most of the villages not serviced by roads trafficable by vehicles (Anonymous,

2002a). Therefore, most of the villages are located about 2-3 days walking distance from the nearest vehicular road. The landscape rises from flat land bordering India in the south to rugged mountainous terrain in the north. Thus the district has climatic zones ranging from a hot subtropical climate in the south, to a cold temperate climate in the north. The district is administratively divided into two regions: Upper Kheng consisting of the four sub-districts of Bardo, Trong, Nangkhor, and Shingkar; and lower Kheng consisting of Ngangla, Bjoka, Phangkhar and Goshing sub-districts. The district shares its southern border with the Indian state of Assam through the two sub-districts of Phangkhar and Ngangla (Figure 4.2).

Figure 4.2 Zhemgang district with its sub-districts and neighbouring districts



Foot-and-mouth Disease has been reported sporadically in Zhemgang with the last recorded outbreak being in July 2002 in Ngangla sub-district which only affected cattle (Anonymous, 2009b). Vaccination against FMD is undertaken mostly in herds located near the roads and in towns. Vaccination is rarely done in villages located far away from the

roads because of the difficulties in transportation as well as in maintaining the cold-chain of the vaccines. Cattle (n=13,669, 90.7%) were the most predominant among the FMD-susceptible species in Zhemgang followed by pigs (n=1,106, 7.3%), goats (n=290, 1.9%), and sheep (n=8, 0.1%) (Anonymous, 2009a).

4.2.2 Source of Data

This study is based on data on FMD outbreaks extracted from the VIS database. Data on the first case, based on the age of the clinical lesions; the geographical location (district, sub-district, and village); species of animal affected; number of cases and deaths in each species; and at-risk population in the sub-districts were collected during a series of disease investigations undertaken by the veterinarians of NCAH and RLDC Gelephu. The XY coordinates for the affected villages were obtained from the Department of Survey and Land Records, Bhutan. A series of follow-up visits were made to the affected herds when one-to-one interviews were held with the farmers. The source of the data is thus multiple: the disease investigation report submitted by officials of the concerned sub-districts, RLDC Gelephu and NCAH. The disease investigations were undertaken from 29 May until 31 December 2007 in Sarpang and from 1 August until 31 December 2007 in Zhemgang. A village recording more than one outbreak within a 1-week period was considered to have only one outbreak because of the close proximity of herds and common management system.

4.2.3 Serotyping and molecular studies

Samples, including fresh vesicular fluid and epithelium from the tongue, buccal mucosa and feet from some infected animals preserved in 50% phosphate glycerol saline, were sent to the WRLFMD for serotyping using the indirect sandwich ELISA (Ferris and Dawson, 1988, OIE, 2008). Virus isolation with subsequent nucleotide sequencing for the VP1

protein was performed at WRLFMD using RT-PCR (OIE, 2008) and a phylogenetic tree was constructed using MEGA version 4 (Tamura *et al.*, 2007) to compare the relationship between the viruses circulating in the country as well as in the region.

4.2.4 Data analysis

Descriptive analyses were carried out using Microsoft Office Excel 2003, and statistical analyses were undertaken using the statistical software SPSS 18 for Windows (SPSS Inc., Chicago, IL, USA) and an on-line epidemiological calculator (<http://epitools.ausvet.com.au>) developed by Sergeant (2009).

Cumulative incidence was calculated to describe the frequency of disease in the population. This was calculated by dividing the total number of FMD-affected animals, during the study period, by the total susceptible population at the beginning of the study period (Dohoo *et al.*, 2003). Case fatality was calculated by dividing the total number of animals that died from FMD by the total number of cases of FMD reported (Dohoo *et al.*, 2003). The 95% confidence intervals for the proportions were calculated using the exact binomial method (Ross, 2003). Spatiotemporal clusters of FMD were identified by using the space-time permutation model of the scan statistic test using SatScan v8.0.1 (Kulldorff and Inc., 2009). The spatiotemporal technique is based on a hypothetical spatiotemporal cylinder at the geospatial coordinates of each location where outbreaks have been reported. The circular base and the height of the cylinder represent the geographical area (spatial) and temporal dimensions, respectively, for each cluster of outbreaks (Kulldorff *et al.*, 1998). The base and height of the cylinder were allowed to vary up to a maximum size equivalent to the inclusion of 50% of the reported outbreaks (Kulldorff *et al.*, 1998). The observed-to-expected ratio was computed within each cylinder and this was used to estimate the likelihood that the cylinder actually represented a cluster of FMD outbreaks. A

Monte Carlo simulation method was used in combination with the scan test to detect significant differences between the observed and expected values per cluster (Kulldorff *et al.*, 1998).

Results of the spatiotemporal analysis generated by SatScan™ were mapped using ESRI™ ArcGIS® v9.3 (ESRI 1999-2008).

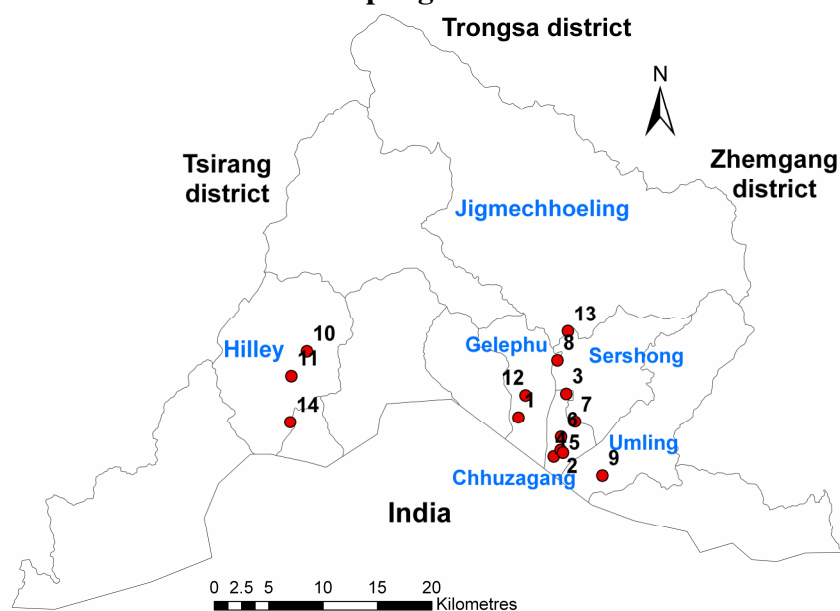
4.3 Results

4.3.1 Disease epidemiology in Sarpang district

4.3.1.1 Spatial distribution

A total of 14 villages in six sub-districts were affected with FMD during the 2007 epidemic (Figure 4.3). Two villages in Gelephu sub-district, namely Puranobusty and Pelrithang, were selected for detailed epidemiological studies based on cost and logistical reasons.

Figure 4.3 Location of villages that reported the first case of FMD in each of the affected sub-districts in Sarpang



[Name of villages: 1: Puranobusty, 2: Barthang, 3: Chaskar, 4: Zombabi, 5: Tshojan, 6: Yueling, 7: Norbuling, 8: Sershong, 9: Chubarthang, 10: Philingtar, 11: Gangatre, 12: Pelrithang, 13: Tshachu, 14: Ranibagan

Note: Numbers represent chronological order of disease outbreaks

4.3.1.2 Temporal distribution

The first case of FMD in Sarpang was recorded on the 29 May 2007 in Puranobusty village in the sub-district of Gelephu. Subsequently, within a 3-month period, the disease was reported in the sub-districts of Chhuzagang, Sershong, Umling, Hilley, and Jigmechhoeling.

The time line for the first case reported in each sub-district is recorded in Table 4.1.

Table 4.1 Time-line for the first diagnosed case of FMD in the affected sub-districts of Sarpang

Date of first case	Sub-districts
29.5.07	Gelephu
20.6.07	Chhuzagang and Sershong
10.7.07	Umling
22.7.07	Hilley
15.8.07	Jigmechhoeling

In order to understand the disease's epidemiology more clearly, the disease incidence in two villages i.e. Puranobusty and Pelrithang in the Gelephu sub-district were closely monitored for three months from the date the first case appeared. These two villages are located approximately 4 km apart.

Disease incidence in Puranobusty village

The number of new cases rose from 6 cases on day 1 to 24 cases by the end of the second week (Figure 4.4). Thereafter, the incidence declined and within one month the incidence had dropped to zero. Thereafter, no more new cases were recorded for approximately one month when six cases were recorded in a new herd on the 11 September. Thereafter, the cases started to decline and by end of October, the disease had completely subsided in this village.

Disease incidence in Pelrithang village

In Pelrithang village, (Figure 4.5), the disease incidence was similar to Puranobusty whereby the number of new cases dropped to zero within approximately a month. In both

villages, the infection resolved by the end of September after which no new cases were reported.

4.3.1.3 Sub-district level cumulative incidence and case fatality pattern

The overall cumulative incidence in cattle and all species was 7.4% (95% CI: 6.8, 7.9) and 6.5% (95% CI: 6, 7), respectively (Table 4.2).

Figure 4.4 Disease incidence in Puranobusty village, Gelephu sub-district.

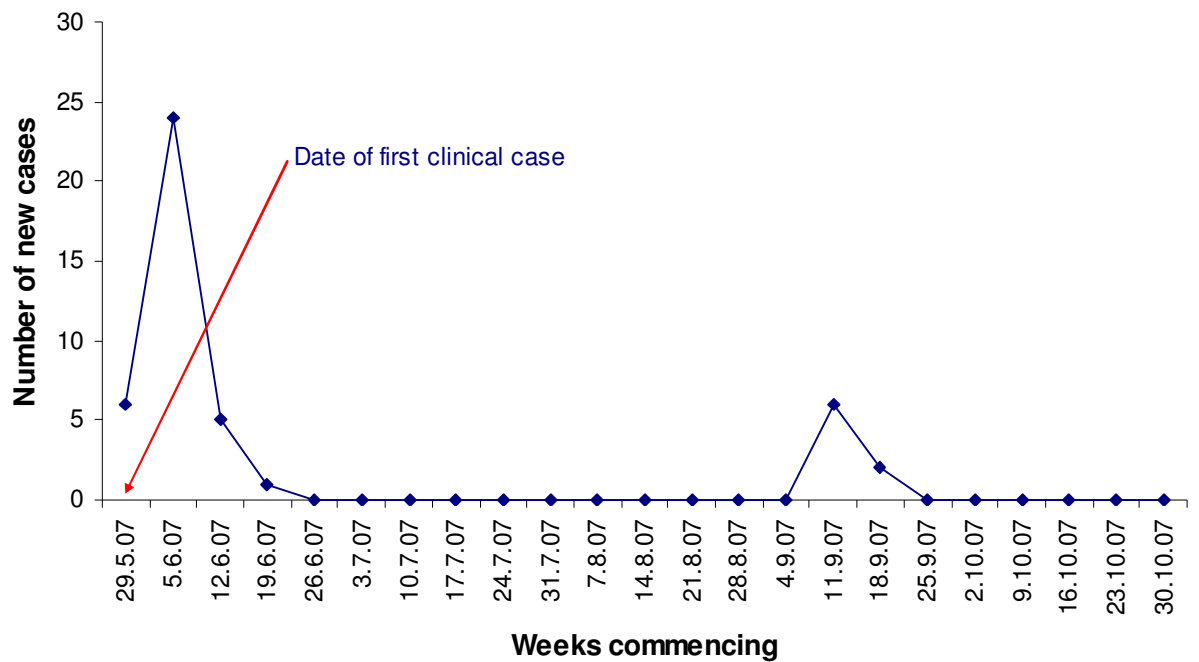
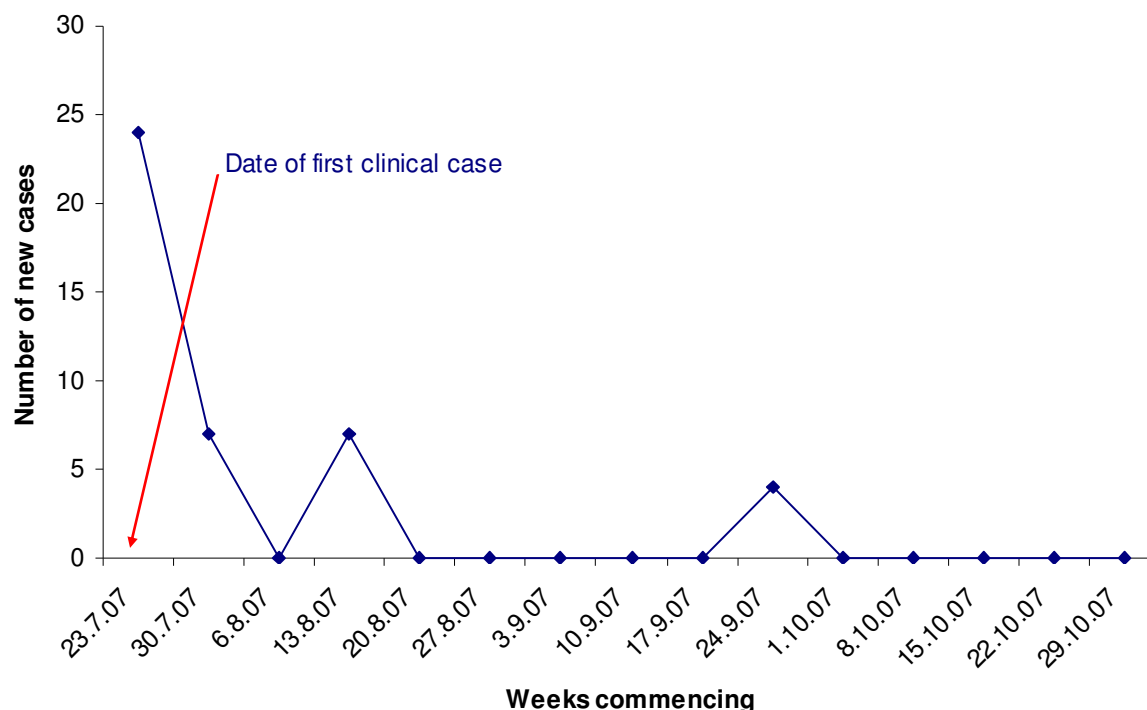


Figure 4.5 Incidence of FMD in Pelrithang village, Gelephu sub-district



There were significant differences ($P < 0.0001$, $\chi^2 = 71.3$, $df = 3$) between the species with sheep recording the highest incidence (9.4%, 95% CI: 5.4, 14.7) followed by cattle (7.4%, 95% CI: 6.8, 7.9), goats (2.1%, 1.4, 3.1), and pigs (1.9%, 95% CI: 0.9, 3.5).

Table 4.2 Cumulative incidence and case fatality for different species in Sarpang district

Species	Population	Total number of cases	Cumulative incidence in % (with 95% CI)	Total number of deaths	Case fatality in % (with 95% CI)
Cattle	8610	633	7.4 (6.8, 7.9)	21	3.3 (2.1, 5.0)
Goats	1319	28	2.1 (1.4, 3.1)	0	0.0 (0.0, 12.3)
Sheep	171	16	9.4 (5.4, 14.7)	0	0.0 (0.0, 20.6)
Pigs	482	9	1.9 (0.9, 3.5)	0	0.0 (0.0, 33.6)
Overall	10582	686	6.5 (6.0, 7.0)	21	3.0 (1.9, 4.6)

The overall case fatality in cattle was 3.3% (95% CI: 2.1, 5). There were no deaths in sheep, goats and pigs in Sarpang.

4.3.2 Disease epidemiology in Zhemgang

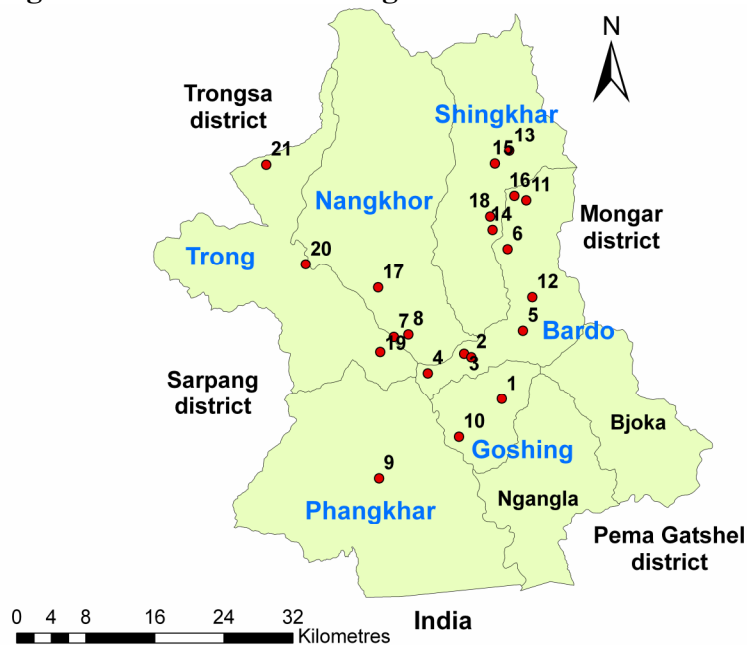
4.3.2.1 Spatial distribution

A total of 21 villages in six sub-districts were affected with FMD (Dorji and Changlo, 2007, Tenzin, 2007) during the 2007 epidemic, as displayed in Figure 4.6. The overall progression of the disease at the sub-district level, defined by the date of occurrence of the first case in each sub-district, is displayed in Figure 4.7 (a-e). By week 8 (19.9.07 to 25.9.07), the epidemic had spread to six sub-districts.

4.3.2.2 Temporal distribution

The first case of FMD in Zhemgang was recorded in the village of Mewagang in the Goshing sub-district on the 1 August 2007 (Dorji and Changlo, 2007). Thereafter, the disease was reported in five other sub-districts (Bardo, Nangkhor, Phangkhar, Shingkhar and Trong). The epidemic lasted until the end of December 2007.

Figure 4.6 Location of villages affected with FMD in Zhemgang



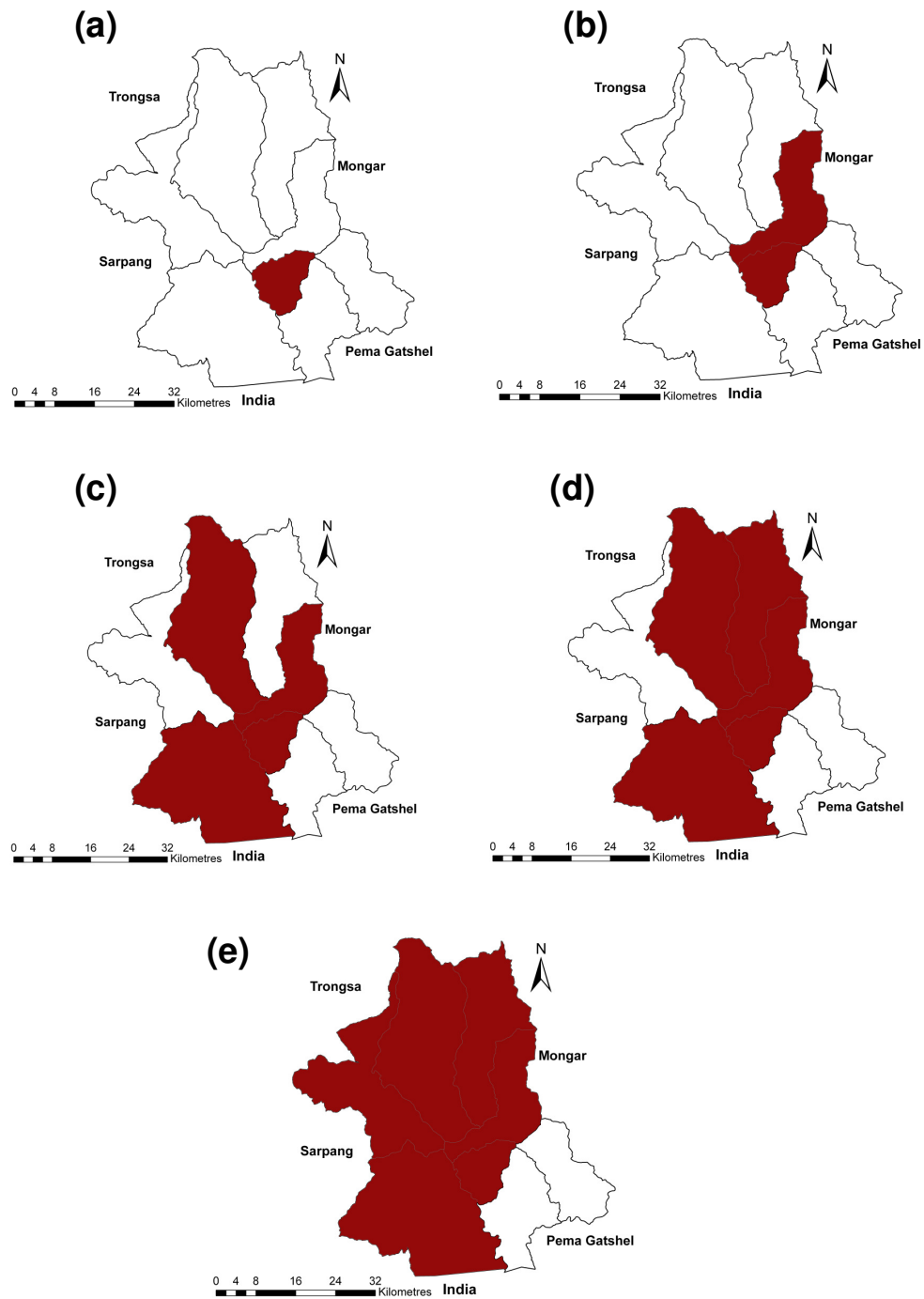
[Name of villages: 1: Mewagang, 2: Relangbi, 3: Digela, 4: Kalamti, 5: Langdurbi, 6: Khomshar, 7: Dunmang, 8: Kamjong, 9: Sansiri, 10: Lamtang, 11: Jawaling, 12: Phulabi, 13: Wamling, 14: Thajong, 15: Radhi, 16: Sameth, 17: Tshaidang, 18: Nimshong, 19: Gomphu, 20: Goling, 21: Trong]

Note: Numbers represent disease outbreaks in chronological order

Detailed data were collected for three sub-districts (Bardo, Nangkor, and Shingkar), accounting for 11 of the 21 affected villages. The cumulative weekly number of cases and deaths (Figure 4.8) in three sub-districts was collected for a period of two months only due to logistical and cost constraints. However, the cumulative total number of cases and deaths in the study area was collected at the end of the study period (Tenzin, 2007).

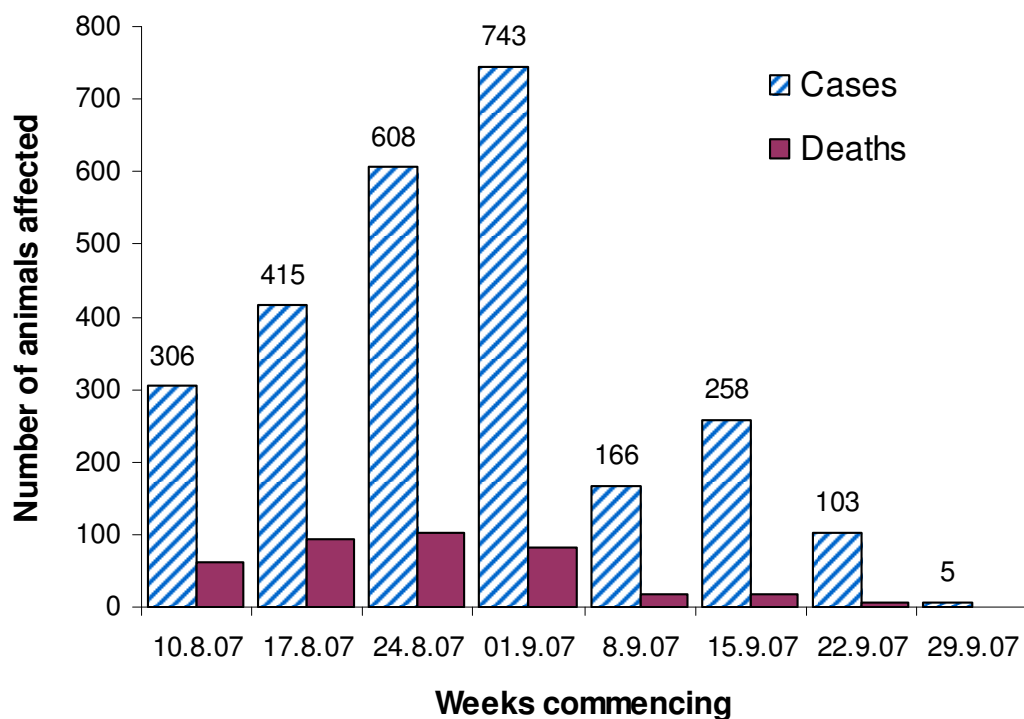
The epidemic peaked in the fourth week (first week of September) after which the number of cases declined rapidly and no further cases were reported after the 5th October.

Figure 4.7 Weekly progression of cumulative incidence of the disease in Zhemgang



Note: (a) Week 1 (1 August-7 August 2007); (b) Week 2 (8 August-14 August 2007); (c) Week 3 (15 August- 21 August 2007); (d) Week 6 (5 September-11 September 2007); (e)Week 8 (19 September-25 September 2007).

Figure 4.8 Cumulative weekly cases and deaths (all species) in three sub-districts of Zhemgang



4.3.2.3 Cumulative incidence and case fatality patterns at the sub-district level in Zhemgang

The overall cumulative incidence in cattle at the sub-district level was 29.3% (95% CI: 28.4, 30.2) and 26.9% (95% CI: 26.1, 27.8) when cases for both cattle and pigs were combined (Table 4.3). The overall cumulative incidence in pigs was 6.4% (95% CI: 5.0, 8.0).

Table 4.3 Cumulative incidence and case fatality for different species in Zhemgang district, Bhutan

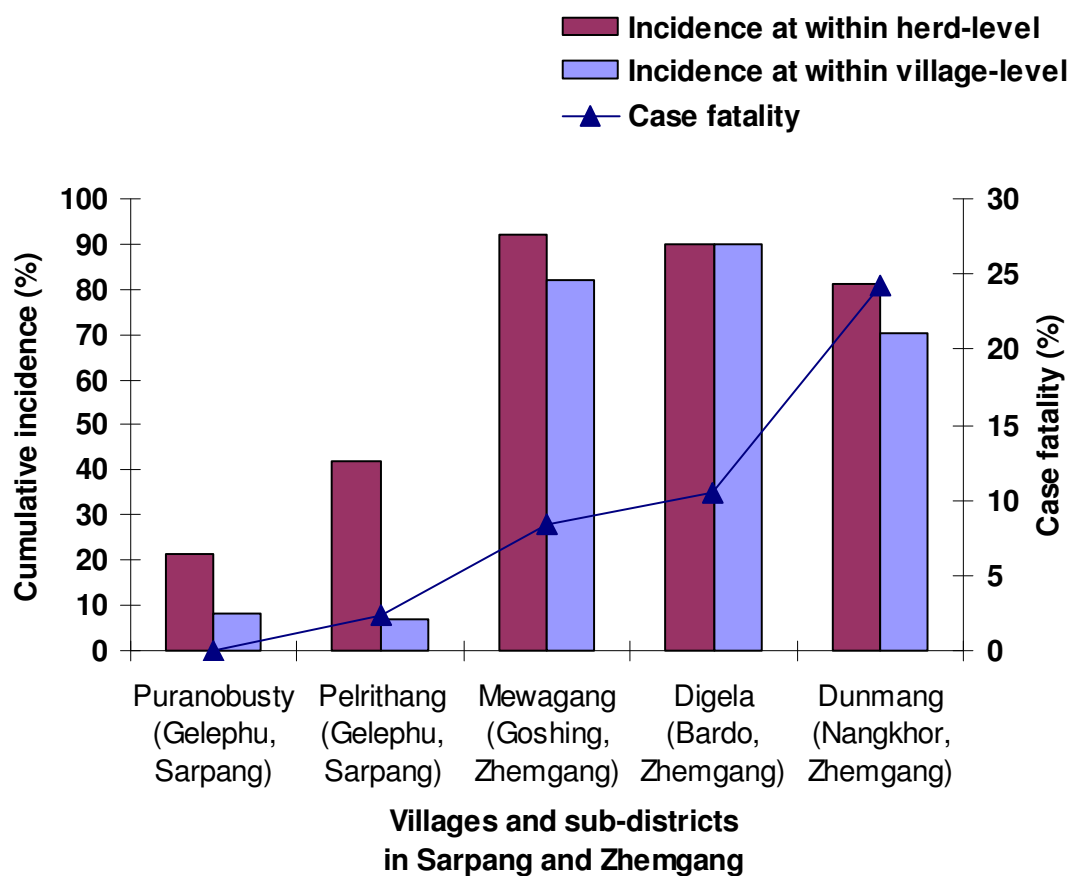
Species	Population	Total number of cases	Cumulative incidence (95% CI)	Total number of deaths	Case fatality (95% CI)
Cattle	9741	2857	29.3(28.4, 30.2)	404	14.1 (12.9, 15.5)
Pigs	1143	73	6.4 (5, 8)	13	17.8 (9.8, 28.5)
Total	10884	2930	26.9 (26.1, 27.8)	417	14.2 (13, 15.5)

The case fatality in cattle (14.1%) was not significantly different ($P=0.38$, $\chi^2=0.8$, $df=1$) from that of pigs (17.8%). There were only 290 goats and 8 sheep (Anonymous, 2009a) in Zhemgang and none were affected during the outbreak.

4.3.3 Comparative cumulative incidence, mortality, and case fatality in Zhemgang and Sarpang

The within-herd and within-village level cumulative incidence, mortality and case fatality patterns in some selected villages in the two districts were compared to identify patterns and differences in the disease epidemiology between the two districts (Figure 4.9). These villages were selected because data on livestock population and disease incidence at the herd and village level were available.

Figure 4.9 Cumulative incidence and case fatality (all species) at the within-herd and within-village level

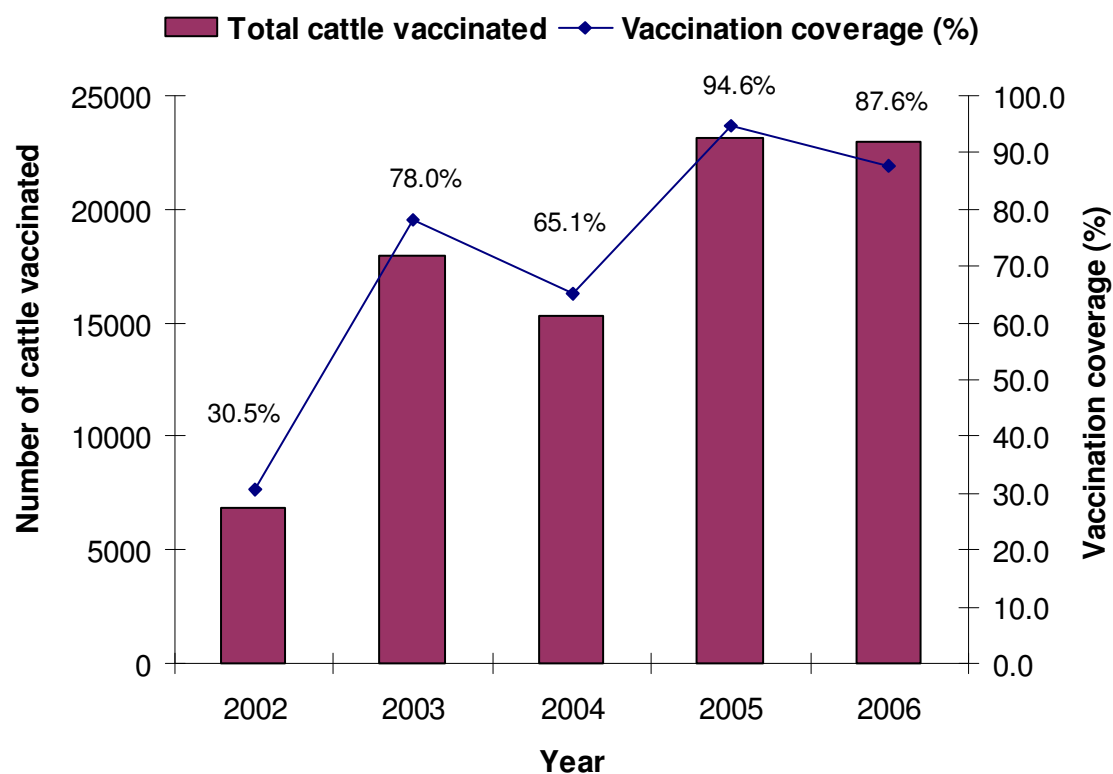


The villages in Zhemgang had a significantly higher incidence of FMD in all species than did villages in Sarpang, both at the within-herd level ($P < 0.0001$, $\chi^2 = 394.2$, $df = 4$) as well as the within-village level ($P < 0.0001$, $\chi^2 = 1129.6$, $df = 4$).

4.3.4 Vaccination status

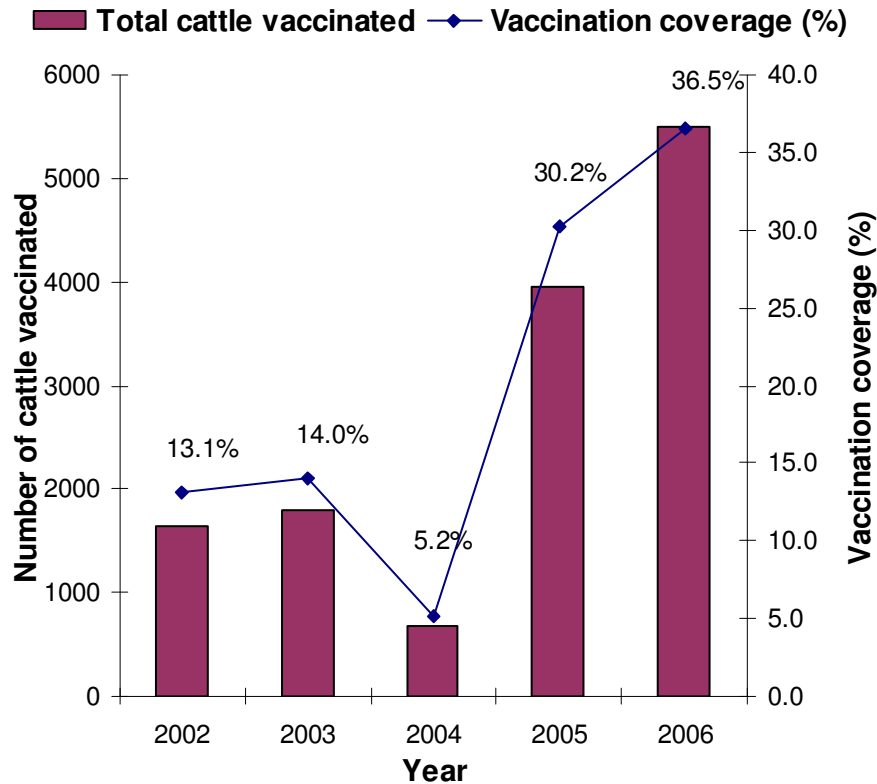
The vaccination coverage for the year 2006 (Figures 4.10 and 4.11) shows that Sarpang had significantly ($P < 0.00001$, $\chi^2 = 11676.1$, $df = 1$) higher vaccination coverage (87.6%) when compared to Zhemgang (36.5%).

Figure 4.10 FMD vaccination coverage in Sarpang between 2002 and 2006



Source: (Anonymous, 2009b)

Figure 4.11 FMD vaccination coverage in Zhemgang between 2002 and 2006



4.3.5 Overall comparative epidemiological features between Sarpang and Zhemgang

Zhemgang recorded a significantly ($P < 0.0001$, $\chi^2 = 1599.9$, $df = 1$) higher cumulative incidence (26.9%; 95% CI: 26.1, 27.8) in all species compared to Sarpang (6.5%; 95% CI: 6, 7). When data for only cattle were considered, the cumulative incidence for Zhemgang (29.3%; 95% CI: 28.4, 30.2) was still significantly higher ($P < 0.0001$, $\chi^2 = 1433.4$, $df = 1$) than that of Sarpang (7.4%; 95% CI: 6.8, 7.9). Cattle in Zhemgang were 5.2 times (95% CI: 4.7, 5.7) more at risk of FMD than those from Sarpang.

The cumulative mortality (all species) in Zhemgang (3.7%; 95% CI: 3.4, 4.1) was significantly higher ($P < 0.0001$, $\chi^2 = 343.7$, $df = 1$) than in Sarpang (0.20%; 95% CI: 0.1, 0.3).

The cumulative mortality for cattle was also significantly higher in Zhemgang (4.2%; 95% CI: 3.8, 4.6) than in Sarpang (0.24%; 95% CI: 0.2, 0.4). The case fatality for cattle in Zhemgang (14.1%; 95% CI: 12.9, 15.5) was also significantly higher ($P < 0.0001$, $\chi^2 = 56.5$,

df=1) than in Sarpang (3.3%; 95% CI: 2.1, 5). A total of 404 cattle and 73 pigs died of FMD in Zhemgang, whereas only 21 deaths were recorded in cattle from Sarpang. There were no deaths in sheep and goats in either district. Calves accounted for approximately 60% of the total deaths in cattle. The fact that 169 adult cattle succumbed to this outbreak is a clear indication of the high virulence of the virus.

4.3.5.1 Clinical features

Cattle in Zhemgang showed severe lesions of FMD (Figure 4.12) compared with those from Sarpang where only mild lesions were seen (Figure 4.13).

Figure 4.12 Severe ulcerations on the dental pad of a cow in Zhemgang (Note the intense swelling of the upper lip)



Figure 4.13 Mild ulcerations on the dental pad of a calf in Sarpang



4.3.6 Spatiotemporal clustering analysis

The time-space permutation method detected four significant ($P < 0.001$) spatiotemporal clusters of outbreaks of FMD in the study area. The four clusters consisted of one primary cluster in Sarpang and three secondary clusters in Zhemgang district (Table 4.4).

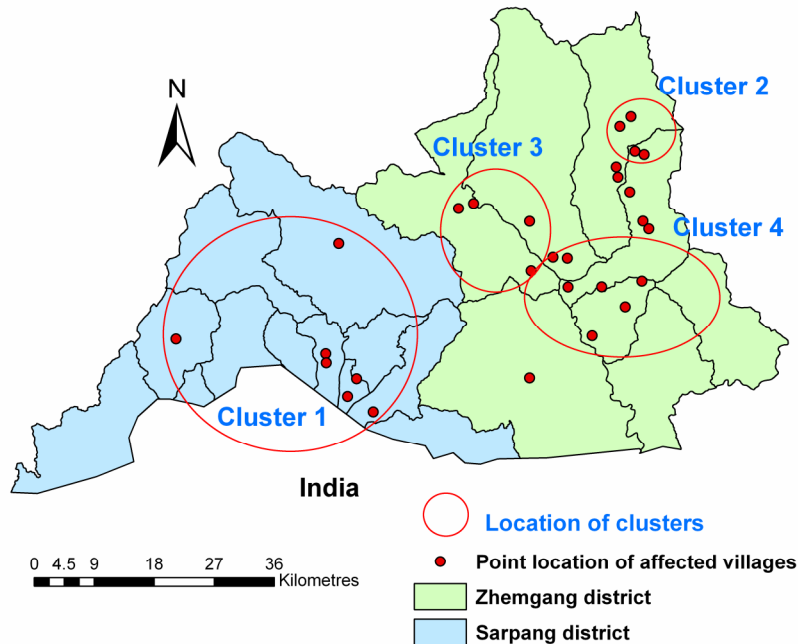
The locations of the spatiotemporal clusters of outbreaks in the region are displayed in Figure 4.14. In Sarpang, spatiotemporal analysis revealed the presence of one highly significant primary cluster ($P = 0.001$) consisting of seven villages in six sub-districts. The cluster had a spatial extension of 22.5 km. In Zhemgang, the spatiotemporal analysis revealed the existence of three highly significant secondary clusters ($P < 0.001$). The spatial extension of the cluster was 13.24 km. The observed-to-expected ratio for FMD outbreaks

within the spatiotemporal clusters was between 1.5 and 9.8 times higher than the levels expected in these districts and sub-districts (data not shown).

Table 4.4 Spatiotemporal clusters (1 May 2007 to 31 December 2007)

Cluster number	District	Sub-districts included in the cluster	Villages included in the cluster	Radius (Km)	Time frame	No of cases	Observed to expected ratio	P-value
1	Sarpang	Gelephu Chhuzagang, Sershong Umling, Hilley, Jigmechhoeling	Puranobusty Perithang Barthang Chubarthang Norbuling Philingtar Ranibagan Tschachu	22.5	1/5/2007 to 31/12/2007	729	3.98	0.001
2	Zhemgang	Shingkar, Bardo	Wamling Radhi, Sameth	5.24	1/9/2007 To 30/9/2007	164	9.88	0.001
3	Zhemgang	Nangkor, Trong	Tshaidang Gomphu Goling Trong	13.3	1/9/2007 to 30/9/2007	128	9.44	0.001
4	Zhemgang	Goshing, Bardo, Nangkor,	Mewagang Relangbi Kalamti Langdurbi Kamjong Lamtang, Dunmang	13.24	1/8/2007 to 31/8/2007	1399	1.54	0.001

Figure 4.14 Spatiotemporal clusters of outbreaks of FMD



4.3.7 Virus serotyping and phylogenetic results

The viruses isolated were of O serotype (Knowles and Wadsworth, 2007). The virus belonged to the PanAsia strain of the ME-SA toptotype which was previously responsible for an explosive pandemic in Asia and Europe from 1998 to 2001 (Knowles *et al.*, 2005). This strain was closely related to the PanAsia strain that circulated in Bhutan during the 2003/2004 outbreaks (http://www.wrlfmd.org/fmd_genotyping/2007/WRLFMD-2007-00140-Bhutan-O.pdf, accessed on 9 November 2009). This PanAsia strain has been cited to be a new lineage (http://www.wrlfmd.org/ref_labs/ref_lab_reports/OIE-FAOFMDLabReport2008.pdf, accessed on 11 December 2009) which is an offshoot of the earlier PanAsia strain, and is referred to as PanAsia-2 lineage (Knowles *et al.*, 2008). The isolate from Sarpang (O/BHU/11/2007) was 100% identical to the isolate from Zhemgang (O/BHU/18/2007) and 99.84% identical to the other isolate from Sarpang (O/BHU/12/2007). The isolate (O/BHU/12/2007) from Sarpang was 99.84% identical to the isolate from Zhemgang (O/BHU/18/2007)

(http://www.wrlfmd.org/fmd_genotyping/2007/WRLFMD-2007-00140-Bhutan-O.pdf, accessed on 28 September 2010).

4.4 Discussion

The study showed contrasting epidemiological features of FMD in two districts with varying levels of disease prevention and control, and livestock management conditions. The disease occurred in an epidemic form resulting in high incidence risks and mortality rates in Zhemgang, whereas it occurred in a relatively milder form in Sarpang, a district where FMD vaccination is routinely undertaken. There was rapid spread of infection and a large number of cases within a week of the index case being reported in Zhemgang as compared to Sarpang where there were significantly fewer cases reported.

The high incidence and case fatality in Zhemgang district could be attributed to many factors including:

- Firstly, the livestock population in Zhemgang was mostly FMD-naïve as they were rarely or never vaccinated against FMD (Dorji and Changlo, 2007, Tenzin, 2007) and there had been no outbreaks of FMD in the affected villages for many years (Anonymous, 2009b). No animals were vaccinated against FMD during the 3 years preceding the outbreak in the sub-district of Goshing (Dorji and Changlo, 2007), where the index case occurred. The overall vaccination coverage for 2006 in the district was only 36.5% of the total susceptible population. The effectiveness of the vaccination programme by itself is questionable given the very scattered and remote location of the herds and difficulties in maintaining the cold-chain requirement of the vaccines. Most of the veterinary centres did not have refrigerators to store vaccines and there was no electricity available in many areas.

- Most of the farmers had never seen FMD before, and consequently, they misdiagnosed the disease and delayed reporting the disease to the nearest veterinary centre (Tenzin, 2007). This led to unrestricted movement of animals and spread of the disease without timely intervention from the veterinary authorities.
- The virus involved in this outbreak has been identified as the highly virulent PanAsia strain which has the ability to cause pandemics, especially in naïve populations. In a study on the impact of FMD outbreaks caused by the PanAsia strain of the O serotype in Savannakhet province of Lao PDR in 1999, Perry *et al.* (2002) also reported a morbidity of 100% in cattle and buffalo and between 30 to 100% in pigs. All the FMD-susceptible species (cattle, buffalo, goats and pigs) showed characteristic clinical signs during this epidemic and mortalities were reported in buffalo, goats and pigs (Perry *et al.*, 2002). The PanAsia strain of the O serotype also reportedly caused characteristic clinical signs in all FMD-susceptible species including cattle, sheep, goats and camels in Mongolia during the 2000 outbreak (Sakamoto and Yoshida, 2002).
- The high incidence and rapidity with which the disease spread could also be due to the lack of biosecurity in the herds and villages as reported elsewhere (Perry *et al.*, 2002). Animals were allowed to graze freely and therefore there was mixing of infected and non-infected animals at watering and grazing areas, resulting in rapid spread of disease.
- The high case fatality could be attributed partly to the poor management and nursing care provided to the sick animals. Most of the sick animals were left to recover on their own without timely intervention of the veterinary services. Consequently myiasis was common in lesions of the nasal and oral cavities. People

in this district are generally poor and most of them live below the poverty line, therefore the sick animals were not provided good nursing care or fed a nutritious diet.

On the contrary, the disease occurred in a much milder form in Sarpang, although the same serotype and strain was involved. This could be due to the following reasons:

- Owing to its endemic status, FMD vaccination is undertaken quite stringently in Sarpang, and since July 2006, bi-annual vaccination has been performed. The veterinary centre has adequate cold chain facilities to store vaccines. A minimum of 80% vaccination coverage was achieved in the years 2005 and 2006 in accordance with the National FMD Control Programme policy (Anonymous, 2009b). In the year 2006, 87.6% of the total susceptible population were vaccinated against FMD serotypes O, A and Asia 1 (Anonymous, 2007c). Therefore, good herd immunity could have prevented large scale spread of the disease in Sarpang (Malaga *et al.*, 1976, Rast *et al.*, 2010).
- The farmers in this district are well aware of FMD and the need to promptly report cases to the nearest veterinary centre. This enabled the veterinary authorities to quickly impose restrictions on the movement of livestock and livestock products following detection of the outbreak. Animal movements have been cited as one of the most common methods for the spread of FMD (Perry *et al.*, 2002, Abila and Foreman, 2006, SEAFMD, 2008, Shiilegdamba *et al.*, 2008, Wongsathapornchai *et al.*, 2008a). In the UK 2001 outbreak, prompt imposition of a nation-wide movement control of animals, within three days of the first case, significantly reduced the geographical spread of the disease (Gibbens and Wilesmith, 2002).

- The very low incidence and mortality, in comparison to Zhemgang, could be also due to the good nursing care provided by farmers to their animals (Dukpa and Pem, 2007a). Farmers in Sarpang mostly rear exotic breeds of cattle (Anonymous, 2009a) and therefore take good care of their animals as compared to farmers in Zhemgang, who rear mostly local breeds and follow an extensive system of farming.

The source of infection in Sarpang was traced to outbreaks that had occurred in a nearby Indian village in Assam. There was anecdotal evidence of outbreaks of FMD in the border village in India one month prior to the outbreak in Gelephu (Dukpa and Pem, 2007b). The cattle in Gelephu possibly contracted the disease whilst grazing common pastures with infected animals from the neighbouring Indian village. Gelephu is the main commercial sub-district and also houses the only slaughter house in the district. Therefore, people from other areas visit this town for purchasing household goods, vegetables and fresh meat. Due to the poor or almost non-existent biosecurity in the infected herds and villages, there is a possibility of both direct and indirect spread of disease to other villages through movement of animals and animal products, as well as indirectly through humans.

It is unlikely that disease transmission within Sarpang could have been through aerosol spread. The period from May to August in Sarpang is characterised by hot and humid weather with maximum temperatures reaching up to 40°C and therefore it is likely that any infective aerosols would have been rapidly inactivated (Hutber and Kitching, 2000). The high incidence in sheep, as compared to cattle, could be a reflection of the fact that these species are rarely vaccinated and can therefore play an important role in the spread of FMD.

The source of the first case in Zhemgang could not be confirmed as there were no reports of the movements of livestock or livestock products into Mewagang village from Sarpang. Farmers in the village reported having seen sick deer and wild boars in their fields and therefore believed that these wild animals could have been the source of infection. Wildlife, such as gazelles (*Procapra gutturosa*), have been suspected to be the source of FMD during the 2001-2003 epidemic in Mongolia, although this claim has not been fully substantiated (Shiilegdamba *et al.*, 2008). Apart from the African buffalo (*Syncerus caffer*), FMDV cannot be maintained in other wildlife species (OIE, 2008) and therefore it is unlikely that wild animals were the source of the outbreak in Zhemgang. It is likely that the disease could have been spread through movements of animals incubating the disease or people or fomites.

The space-time permutation method of the scan statistics identified a primary cluster in Sarpang district and three secondary spatial-temporal clusters in Zhemgang district. The observed-to-expected ratio for FMD outbreaks within the temporal-spatial clusters was between 1.5 and 9.88 times higher than the background level expected in these districts and sub-districts. The spatial extension of the largest cluster, detected in Sarpang, was 22.5 km. This suggests that a buffer zone larger than 22 km would be required for effective control of future FMD outbreaks in the country. However, given the mountainous terrain and scattered location of villages and herds, it may not be feasible to implement a buffer zone of 22 km or larger. Three of the clusters had a spatial spread greater than the currently recommended radius for the control zone (10 km) for FMD used in Bhutan (Anonymous, 2005). This suggests that the currently used control and surveillance zone is not sufficient to control the disease from spreading. There is virtually no biosecurity implemented in the infected villages and herds and therefore, it is highly likely that the high infection rate in the clusters could be attributed to the increased movement of animals or animal products.

Similar findings have been reported in Mongolia (Shiilegdamba *et al.*, 2008) where local movement of animals and transportation were suggested as possible modes of transmission within clusters of outbreaks.

Phylogenetic analysis showed that the viruses isolated from Zhemgang were identical to those from Sarpang. Consequently, based on the timeline, the infection could have spread from Sarpang to Zhemgang, although the route of transmission is not clear. Most of the affected villages in Zhemgang are linked by only one mule track and therefore it is likely that the disease could have spread to other villages through movement of livestock, livestock products and contaminated humans and via other fomites. Most of the farmers reported seeing crows feeding on the saliva, inter-digital lesions, and blood debris in the nostrils of affected animals. Farmers also reported seeing large scale movement of crows between villages and this was also thought to have contributed to the spread of disease between the villages.

The findings from this study may be considered to further strengthen the existing FMD control programme.

- For instance, there is a need to reconsider the 10-km radius for ring vaccination currently used as this may not be large enough to prevent the spread of disease.
- There is also a need to build-up herd immunity in Zhemgang district through a sustained vaccination programme. This should be done in a campaign form in order to minimize vaccination failure. However, vaccination will protect only against the particular serotypes and sub-types that are included in the vaccine. Animals will be susceptible to new serotypes and sub-types that are not included in the vaccine. There is a need to supply kerosene/solar powered refrigerators to the remote centres for effective storage of vaccines.

- Farmers residing in remote sub-districts need to be made aware of the clinical signs and lesions of FMD so that in the event of future outbreaks, they could recognise the disease and report it immediately to the nearest livestock centre. The farmers should also be informed about basic biosecurity measures to be undertaken to prevent the spread of disease between herds and villages.
- There is a need to continue bi-annual vaccination in the high risk zones such as Sarpang. The benefit of sustained vaccination was evident from the recent outbreak in Sarpang where the disease spread was limited.

In conclusion, the study showed contrasting epidemiological features of FMD in two districts with varying levels of disease control. The study highlights the ability of the FMD virus, particularly the PanAsia strain of the O serotype, to cause unprecedented morbidity and mortality, especially in a naïve population.

The study also highlights the benefits of maintaining good herd immunity in a susceptible population, through adequate vaccination coverage, to minimize the severity of infection and limit the spread of disease from infected to non-infected herds. Information generated from such comparative studies can be used to effectively manage FMD in Bhutan. Similar comparative epidemiological studies need to be undertaken in other FMD-endemic countries to provide additional information on how the PanAsia strain of the O serotype behaves under different control mechanisms.

There is a need to understand the herd-level management system of animals and risk factors that are associated with the occurrence and spread of FMD in the villages. A study investigating the herd-level risk factors in the sedentary herds of Bhutan is presented in the next chapter.

Risk factors for foot-and-mouth disease in the sedentary livestock herds in selected villages in four regions of Bhutan

This Chapter has been published in a peer-reviewed journal:

Dukpa, K., Robertson, I.D., Edwards, J.R., Ellis, T.M., Tshering, P., Rinzin, K. and Dahal, N. (2011) Risk factors for foot-and-mouth disease in sedentary livestock herds in selected villages in four regions of Bhutan. *New Zealand Veterinary Journal* **59** (2), 51-58.

5.1 Introduction

Despite a national control programme, FMD continues to occur in Bhutan (Anonymous, 2009b) mainly due to a lack of information on the disease's epidemiology, in particular a deficiency in information on the reasons as to why some herds are affected while others are not. Since livestock production is predominantly extensive in Bhutan, it is likely that specific management procedures will predispose animals to diseases such as FMD. Therefore, there is a need to know, in detail, the livestock husbandry practices adopted by farmers in order to understand the risk factors associated with the occurrence and spread of FMD. It is essential to understand the risk factors at the herd (Bronsvort *et al.*, 2004a) and village-level (Cleland *et al.*, 1996) that are associated with the occurrence and transmission of FMD before developing a disease control programme.

Questionnaire surveys have been successfully used in FMD-endemic countries including Thailand (Cleland *et al.*, 1996), Cameroon (Bronsvort *et al.*, 2004a), Ethiopia (Megersa *et al.*, 2008) and Ecuador (Lindholm *et al.*, 2007), to identify and understand risk factors associated with the occurrence of FMD in those countries.

This is the first study investigating risk factors associated with FMD in Bhutan and the study was performed with the following objectives:

- To understand the livestock husbandry practices prevalent in the area
- To identify and quantify the herd-level factors associated with the occurrence and spread of FMD in the sedentary herds of Bhutan.

5.2 Materials and methods

5.2.1 Study design

A cross sectional survey was conducted between March and May 2009 wherein questionnaires were administered to livestock farmers in conjunction with a seroprevalence study in four districts of Bhutan. The survey was conducted amongst livestock farmers who practice sedentary farming, i.e., do not undertake long-distance seasonal movement with their animals. For the purpose of this Chapter, each household is considered as a herd.

5.2.2 Study area

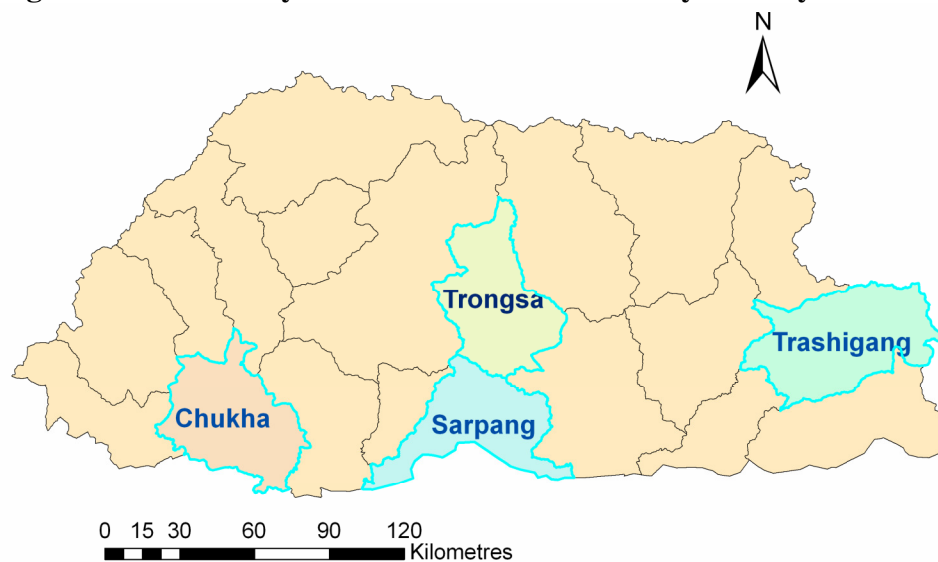
The study was undertaken in the four districts of Sarpang (south), Trongsa (central), Trashigang (east) and Chukha (west) representing the four agro-ecological regions of the country (Figure 5.1). These districts represented the varying agro-climatic zones from wet sub-tropical to cool temperate climate. In each of these districts, those sub-districts which were inaccessible were not included in the study due to time, cost and logistical

constraints. Even within a selected sub-district, villages which were remote and inaccessible were excluded from the study for the same reasons.

5.2.3 Sample size

With an assumed herd-level prevalence of 50%, 800 villages in the four selected districts, an average of 25 households per village, a 20:1 cost ratio of village to household, a precision of $\pm 8\%$ and 95% confidence intervals, and a within-village and between-village variance of 0.1, the programme Survey tool box version 1.0 beta, (Cameron, 1999), gave a two-stage sample size of 60 villages and 5 households per village. In the actual study a total of 383 households were interviewed from 80 villages.

Figure 5.1 The study area for the risk factor analysis study



5.2.4 Sampling methods

A multi-stage, stratified, probability proportional to size random sampling method was undertaken to select the villages and the households for undertaking the seroprevalence study (Chapter 6). The farmers selected for the seroprevalence study were also interviewed for the risk factor analysis, the results of which are reported in this chapter.

5.2.5 Questionnaire

A questionnaire (approved by the Murdoch University Human Ethics Committee) was used in this study. The questionnaire was written in English since the enumerators were well versed in English. The questionnaire was pre-trialled on 5 farmers each from Tsirang and Sarpang districts, modified slightly and a final version produced. The questionnaire (Appendix 1) contained questions on various aspects of livestock husbandry and management practices, including the occurrence of clinical signs of FMD in the farmers' herds over the preceding five years. A core team consisting of senior animal health staff from NCAH and the respective RLDCs accompanied the researcher in each of the surveyed districts. A one-day workshop was organized in each district wherein all the livestock extension officials working in the district, as well as the core team, were briefed about the objectives of the survey and the materials and methods to be used. The questionnaire was explained in detail, page-by-page, to all the enumerators. Thereafter, all the enumerators were given hands-on practice in completing the questionnaire by conducting a mock-survey exercise in a nearby village. All completed questionnaires were checked for any ambiguities and doubts were cleared. Photographs of FMD, depicting key clinical signs and lesions, were shown to the farmers before the start of the interview. The enumerators were instructed beforehand not to reveal the purpose of the survey to the farmers so that the farmers would not have a preconceived notion about the disease. Once the photographic introduction was completed, the farmers were then briefed about the main purpose of the survey. For the purpose of studying the general livestock husbandry practices adopted by the farmers, all questionnaires were considered as valid. For the purpose of risk factor analysis, only those questionnaires in which a farmer correctly identified a picture of FMD or could name some of the main clinical signs of FMD were included for analysis. This was to ensure that the farmer had some knowledge about the

disease and the researchers were confident that the questionnaires could be included in the risk factor analysis. Respondents were considered as literate if they could read or write in either English or Bhutan's national language, *Dzongkha*. Each questionnaire took between 15 to 20 minutes to administer.

5.2.6 Data analysis

Data management and statistical analyses were undertaken as described previously (Chapters 3 and 4). Univariable analyses, using Chi-square tests were used to measure statistical significance of association between the hypothesized risk factors (predictor variable) and the outcome variable ("farmer-diagnosed FMD"). Odds ratios and their 95% confidence intervals were used to measure the strength of association between risk factors and disease. The Fisher's exact test was used to report statistical significance of association when one of the cells in a 2x2 table had outcome or expected counts less than 5. Only variables which had P-values <0.25 in the univariable analyses were included in the subsequent multivariable logistic regression model (Hosmer and Lemeshow, 2000, Noordhuizen *et al.*, 2001). A backward conditional method, with the sequential manual removal of variables based on a lack of statistical significance and biological plausibility, was used to produce the best fit model (Baker *et al.*, 1999). For the factors to remain in the final model, a significance level was set at the likelihood ratio of P-value of 0.05 for entry and 0.10 for removal.

The variables included in the final model were checked for collinearity as described by Pallant (2005). Variables with tolerance values greater than 0.1 were considered not to be correlated with other variables and were therefore retained in the final model. The predictor variables in the final model were also assessed for potential interactions. Each

interaction was added to the model and the significance was assessed in the same way as for the explanatory variables. The overall model fit was assessed using several tests: the Hosmer-Lemeshow χ^2 statistic (Hosmer and Lemeshow, 2000), Omnibus tests of model coefficients, Cox & Snell R Square and Nagelkerke R Square (Pallant, 2005).

5.3 Results

5.3.1 Profile of study population

A total of 383 farmers were interviewed from 80 villages located across 28 sub-districts of the four selected districts (Table 5.1).

Table 5.1 Summary of the sub-districts, villages and respondents that took part in the risk factor study

Districts	Sub-districts	Villages	Total farmers interviewed	Percentage of all respondents
Sarpang	9	19	100	26.1%
Trongsa	4	19	94	24.5%
Trashigang	8	20	98	25.6%
Chukha	7	22	91	23.8%
Total	28	80	383	100%

The distribution of the study population (farmers) in terms of gender, literacy, income source, and land holdings is summarized in Table 5.2. More males were interviewed (59%, 226/383) than females (41%). Most (74%, 284/383) respondents were not literate.

When asked about their main source of income, most farmers reported agriculture (58%, 220/383) followed by livestock (34%, 131/383), and others 8% (32/383) such as casual employment, businesses or money sent by their children. The mean area of land owned per household was 1.66 hectares (range: 0 – 10.11 hectares) and a household was found to consist of, on average, 5.5 persons (range: 1 – 15). On average, each respondent had spent

36 years living in their current village (range: 1 - 82 years) and had been undertaking livestock farming activities for an average of 27 years (range: 1-70 years).

Table 5.2 Profile of the survey respondents

Variables	Chukha n (%)	Sarpang n (%)	Trashigang n (%)	Trongsa n (%)	Total n (%)
Gender					
Male	63 (69)	78 (78)	56 (57)	29 (31)	226 (59)
Female	28 (31)	22 (22)	42 (43)	65 (69)	157 (41)
Literacy					
Literate	38 (42)	21 (21)	24 (24)	16 (17)	99 (26)
Illiterate	53 (58)	79 (79)	74 (76)	78 (83)	284 (74)
Main source of income					
Agriculture	47 (52)	52 (52)	80 (82)	41 (44)	220 (58)
Livestock	40 (44)	38 (38)	10 (10)	43 (46)	131 (34)
Other	4 (4)	10 (10)	8 (8)	10 (11)	32 (8)

5.3.2 Livestock husbandry system

The livestock farming system adopted in the study area was predominantly subsistence-orientated with small scale rearing of cattle and other species for multiple purposes. While all respondents reared cattle, only 20% (76/383) and 13% (48/383) of the farmers also reared small ruminants or pigs, respectively, along with their cattle. Each household owned a mean of 8.4 cattle (SD 5.4), 0.3 sheep (SD 2.3), 0.9 goats (SD 2.3) and 0.3 pigs (SD 1.0). Cattle were reared mainly for dairy production (67%, 257/383), as a source of manure for improving the fields (20%, 44/383), for draught purposes (11%, 78/383) and for sale

during times when money was needed (1%, 4/383). However, these were not exclusive and farmers were found to use cattle for multiple purposes.

Cattle were the predominant species reared, constituting 61.1% (3223/5275) of the total livestock population (Table 5.3). Poultry (27.5%, 1449/5275) were the next most common species reared followed by goats (6.6%, 349/5275), pigs (2.3%, 119/5275), sheep (2%, 104/5275), and buffalo (*Bubalus bubalis*) (0.2%).

Table 5.3 Distribution of livestock in the study area

Species	Chukha	Sarpang	Trashigang	Trongsa	Total	Percent of all animals
Cattle	779	903	662	879	3223	61.1
Buffalo	0	12	0	0	12	0.2
Sheep	31	33	0	40	104	2.0
Goats	141	198	10	0	349	6.6
Pigs	68	30	14	7	119	2.3
Horses	3	10	3	3	19	0.4
Poultry	361	849	178	61	1449	27.4
Total	1383	2035	867	990	5275	100.0

During the day, farmers either tethered their cattle near their house (23%, 90/383) or allowed them to move freely around their housing compound (73%, 281/383) when not being grazed away from home. These were not exclusive as within a herd, while some cattle were tethered, others were kept untethered. A small proportion of the farmers (9%, 35/383) kept their cattle housed in a shed at all times during the day. However, these practices were found not to be mutually exclusive and often, even within the same herd, some cattle (high productive animals and calves) were kept indoors whereas others (low productive animals) were allowed to graze freely. In contrast, most farmers kept their small ruminants (51%, 39/76) and pigs (96%, 46/48) permanently confined to a shed or pen (Figures 5.2 and 5.3).

During the night, most of the farmers (80%, 305/383) confined their cattle to sheds, although some (21%, 80/383) kept them tethered outside and a few (3%, 12/383) allowed their animals to wander around their fields. Sheep and goats (96%, 73/76) and pigs (96%, 46/48) were almost invariably found to be kept in sheds or pens at night.

The animals were managed under an extensive feeding system whereby most farmers allowed their animals to graze freely in their village and adjacent forest areas (Figure 5.4).

The animals were fed a variety of materials including commercial feed, crop by-products, kitchen wastes, improved pastures and the leaves from fodder trees. The crop by-products fed consisted of maize grain, rice straw, bran or husk, and maize plant (stalk).

The animals' drinking water was sourced from a reticulated source (tap), rivers, streams, spring or from irrigation sources.

5.3.3 Prevalence of FMD

Based on the responses of farmers, 62% of the villages (95% CI: 50.4, 72.7) and 24% of the herds (95% CI: 20.1, 29.3) had at least one outbreak of FMD within the preceding 5-years. There were significant differences between the four districts for both the village-level (Table 5.4 - $P=0.04$, $\chi^2=8.12$, $df=3$) as well as at the herd-level prevalence ($P=0.03$, $\chi^2=9.02$, $df=3$). Trongsa had the highest prevalence both at the herd (31.1%, 95% CI: 21.8, 41.7) and village-level (84.2%, 95% CI: 60.4, 96.6).

5.3.4 Disease epidemiology

5.3.4.1 Disease incidence at village level

A total of 156 respondents reported seeing FMD in their villages during the 5-years preceding the survey. The severity of FMD in the affected villages was assessed based on the number of cattle that had been affected with FMD in the most recent outbreak. When asked about the proportion of cattle in the affected village showing clinical signs of FMD,

approximately 12% (19/156) of the respondents reported clinical signs in all cattle in their villages. The majority (55%, 56/156) reported that only half of the cattle population in their village showed clinical signs of FMD.

Figure 5.2 Goats being kept in a shed and stall-fed



Figure 5.3 A pig housed in a sty made of bamboo and timber



Figure 5.4 Cattle grazing in the forest in Sarpang district



Table 5.4 Farmer-diagnosed prevalence of FMD in Bhutan

District	No. of villages affected with FMD/No. surveyed*	Prevalence (%) at village-level (95% CI)	No. of herds affected with FMD/No. surveyed*	Prevalence (%) at herd-level (95% CI)
Chukha	14/22	63.6 (43.5, 82.2)	17/87	19.5 (11.8, 29.4)
Sarpang	11/18	61.1 (35.7, 82.7)	29/94	30.9 (21.7, 41.2) ^b
Trashigang	8/20	40.0 (19.1, 63.9) ^a	13/84	15.5 (8.5, 25.0) ^{b, c}
Trongsa	16/19	84.2 (60.4, 96.6) ^a	28/90	31.1 (21.8, 41.7) ^c
Total	49/79	62.0 (50.4, 72.7)	87/355	24.5 (20.1, 29.3)

* Number after validation of each questionnaire; Letters with the same superscripts are significantly different ($P < 0.05$)

Most farmers (51%, 79/156) believed that the source of FMD in their villages was from the movement of infected animals. Other sources of FMD identified were the movement of meat from infected villages (22%, 34/156), movement of dairy products (7%, 11/156), the illegal importation of meat from India (3.2%, n=5), mixing of village livestock with livestock from India (3%, 5/156), and wildlife (1%, 2/156).

5.3.4.2 Disease incidence at the herd-level

Of the 156 respondents who reported seeing FMD in their village, only 87 also reported FMD in their herds. The respondents reported occurrence of FMD in their herds at 6 to 10 year intervals (24%, 21/87), once every 2 to 3 years (23%, 20/87), once every 4 to 5 years (22%, 19/87) or once a year (13%, 11/87).

5.3.4.3 Species susceptibility

Cattle were the most common species affected with FMD, being recorded in all the affected households (herds) whereas only one household reported the occurrence of

clinical signs in goats. No clinical signs were observed by farmers in pigs from any villages during the 5-year period preceding the survey.

5.3.4.4 Morbidity and case fatality in cattle

A total of 613 cases of FMD were reported in cattle in the study area with a median of 5 cases per herd (range: 1-45). The overall morbidity was 67.9% (95% CI: 64.7, 70.9) when the number of cases were compared with the total cattle population in the herd at the time of survey (Table 5.5). There was a significant overall difference in the morbidity between the districts ($P < 0.01$, $\chi^2 = 57.4$, $df = 3$), however the morbidity in Trongsa was similar to that of Sarpang. The overall case fatality was 9.3% (95% CI: 7.1, 11.9). The case fatality was similar in all four districts.

5.3.4.5 Disease control measures

In response to occurrence of FMD in their herds, farmers adopted various measures to control the disease. Most (86% - 75/87) respondents reported cases of FMD to the nearest veterinary centre to seek assistance from the government for disease control.

Forty-six percent (40/87) of the respondents reported having used ethno-veterinary medicine, such as honey mixed with buckwheat (*Fagopyrum esculentum*), and molasses for the treatment of animals affected with FMD. Farmers, especially in Sarpang and Chukha, reported keeping their FMD-affected cattle in mud to hasten the wound healing and to avoid infestation with maggots in the lesions. Approximately 13% (11/87) of the farmers left their FMD-affected animals to graze freely in the village. None of the farmers reported selling their animals when they were infected with FMD.

Table 5.5 Morbidity and case fatality patterns in cattle

District	No. of cases	Cattle population	No. of cattle dead	Morbidity (% with 95% CI)	Case fatality (% with 95% CI)
Chukha	135	157	18	86.0 (79.6, 91.0) ^a	13.3 (8.1, 20.3) ^b
Sarpang	199	300	18	66.3 (60.7, 71.7) ^{a,b}	9.0 (5.4, 13.9) ^b
Trashigang	42	102	7	41.2 (31.5, 51.4) ^a	16.7 (7, 31.4) ^b
Trongsa	237	344	14	68.9 (63.7, 73.8) ^{a,b}	5.9 (3.3, 9.7) ^b
Total	613	903	57	67.9 (64.7, 70.9)	9.3 (7.1, 11.9)

^a Significant differences ($P < 0.05$), ^b Non-significant differences ($P > 0.05$)

5.3.5.6 Impact of FMD

The respondents reported that the occurrence of FMD in their herd resulted in a range of impacts to their livelihoods. The majority (90%, 78/87) reported having suffered loss of income due to reduced milk production after an outbreak of FMD in their herd and 60% (52/87) of farmers reported a loss of draught power in affected draught animals.

Farmers (34%, 30/87) also reported having to spend extra time looking after sick animals and losing calves (17%, 15/87) during the most recent outbreak of FMD in their herd. The mean number of cattle owned (10.4 ± 7.4 SD) in infected herds was significantly higher ($P = 0.009$, Mann-Whitney U test) than that of non-infected herds (7.9 ± 4.6 SD).

5.3.5 Vaccination profile

The majority (88%, 313/355) of farmers reported having their cattle vaccinated against FMD during the 5-years preceding the survey. Of these, most farmers (78%, 245/313) vaccinated their cattle once a year compared with only 19% (60/313) who had their animals vaccinated twice a year (Table 5.6). The majority of farmers (86%, 269/313) reported vaccinating their entire herd (other than cows in advanced pregnancy or young

calves less than 3 months of age), compared with 8% (26/313) of farmers who vaccinated half their herd and 3% (11/313) who vaccinated only a few animals in their herd.

Table 5.6 Vaccination profile in the study area (Number of respondents)

Vaccination frequency	Chukha	Sarpang	Trashigang	Trongsa	Total	Percent of total
Twice a year	2	55	3	0	60	19.2
Once a year	83	33	56	73	245	78.3
Once every 2-3 years	0	1	0	0	1	0.3
Couldn't remember	0	2	1	4	7	2.2
Total	85	91	60	77	313	100

Of the farmers (n=313) who vaccinated, the majority (99.4%, n=311) reported that the vaccine protected their animals against FMD. Farmers reported that the benefits of vaccination included not having any outbreaks (41%), early recovery of affected animals (30.1%) and animals not getting the disease even though other animals in the village were infected (29.2%).

5.3.6 Movement of livestock and livestock products

Most farmers (86%, 331/383) allowed their animals to graze away from their home (Figure 5.5). Of those farmers who sent their livestock for grazing, nearly all (98%, 324/331) sent cattle for grazing compared with only 29% (22/76) for owners of small ruminants. Grazing in the forest was the most common (87%, 287/331) followed by paddy fields (23%, 77/331) and community grazing grounds (21%, 69/331). These were again not exclusive and farmers allowed their animals to graze in all these areas at some point of time.

Figure 5.5 Cattle returning back after grazing in the forest/paddy field in the late afternoon in Sarpang district



The majority of farmers (96%, 318/331) cited a shortage of feed/fodder as the reason for sending their animals for grazing. Having a large herd size (28%, 92/331) and an inability to buy feed (22%, 74/331) were also cited as other reasons for sending animals for grazing. Animals were sent for grazing for a mean of 8.8 months a year (\pm SD=4.5). Most herds (83%, 276/331) that were sent for grazing mixed with other herds within the same village and approximately one-third (33%, 109/331) of the herds mixed with herds from other villages when at the grazing areas. In those herds where sheep and goats were available, only one-third of the farmers (36%, 25/69) allowed their cattle to graze together with their sheep and goats.

The majority of farmers in the study population either borrowed (75%, 288/383) or lent (73%, 278/383) bullocks for tilling of farmland to other farmers within their village.

Less than half of the farmers (42%, 161/383) purchased animals from their own village compared with 27% (105/383) and 13% (51/383) who purchased them from other villages

within the same district or from a different district, respectively. Twenty-seven percent of the farmers didn't purchase any livestock. However, these were not mutually exclusive as some farmers purchased animals from different sources.

Approximately half of the farmers (48%, 183/383) sold animals. Of these, the majority (67%, 123/183) sold them in their own village compared with 47% (87/183) and 14% (26/183), who sold them to farmers from other villages and other districts, respectively.

A large number of farmers (85%, 325/383) sold livestock products. The majority (91%, 297/325) of these sold processed dairy products such as cheese and butter, 33% (108/325) sold milk, 22% (71/325) sold meat and only one farmer sold wool.

Approximately half (47%, 179/383) of the farmers in the study area purchased dairy products for home consumption from within their village, with no farmers buying dairy products from outside their village. However, 74% (282/383) of the farmers reported having purchased some meat from other villages. In contrast only 10% (38/383) purchased all their meat from within their own village.

The majority (93%, 355/383) of the respondents could recognise the clinical signs or lesions of FMD when a picture of FMD was shown. Most farmers (87%, 332/383) could recall some of the key clinical signs of FMD. Similarly, the majority of respondents (84%, 320/383) understood some of the important disease control measures that needed to be applied in the event of an outbreak of FMD in their herds or villages. When asked to name some of the wild animals susceptible to FMD, the majority (68%, 262/383) correctly identified the names of local wild animals that were susceptible to infection.

5.3.7 Risk factors for farmer-diagnosed FMD in Bhutan

5.3.7.1 Results of the Univariable analysis

A total of 44 predictor variables, based on biological plausibility, were screened for association with the outcome variable, “farmer-diagnosed FMD in Bhutan”. Of these, 35 had P-values less than 0.25 and were therefore included in the multivariable logistic regression model (Table 5.7).

The final logistic regression model for “farmer-diagnosed FMD in Bhutan” is summarised in Table 5.8. The overall model fit was assessed by the Hosmer-Lemeshow test ($\chi^2=3.324$, $P=0.853$, $df=7$) and Omnibus tests of model coefficients ($\chi^2=240.98$, $P<0.0001$, $df=7$) (Pallant, 2005). Between 50.1% and 74.1% variation in the dependent variable could be explained by the set of predictor variables in this model as tested by the Cox & Snell R Square test.

5.3.7.2 Results of multivariable logistic regression

Those cattle herds mixing with more than 6 other herds within the same village were 5.3 times (95% CI: 2.18, 12.89; $P<0.0001$) more likely to have had FMD than those that mixed with less than 6 herds. The odds of having FMD in a herd increased substantially (OR=39.2; 15.2, 101.08; $P<0.0001$) when cattle mixed with herds from other nearby villages as compared to those that didn't mix. Farmers who sent their animals for grazing in the forest were 3.1 times (95% CI: 1.2, 7.4; $P=0.014$) more likely to report FMD in their herds than those who didn't. Farmers who fed kitchen wastes to cattle were 14.1 times (95% CI: 5.6, 35.2; $P<0.0001$) more likely to report FMD in their herds than those who didn't. Farmers who kept their cattle always housed in a shed during the day were less likely (OR=0.033; 95% CI: 0.001, 0.83) to report FMD in their herds as compared to those who didn't. Similarly, farmers who kept their cattle always housed at night were less likely (OR=0.29; 95% CI: 0.10, 0.82) to report FMD in their herds than those who didn't.

Table 5.7 Univariable analysis for “farmer-diagnosed FMD in Bhutan”.

Variable name	Answer	No. +ve/total (% FMD positive)	Odds ratio (95% CI)	P-value
Management and husbandry related predictor variables				
Does the farmer own sheep or goats along with cattle?	Yes	20/79 (25.3)	1.0 (0.6, 1.8)	0.85
	No	67/276 (24.3)	1.0	
Does the farmer own pigs along with cattle?	Yes	8/45 (17.8)	0.6 (0.2, 1.4)	0.17 ^a
	No	79/310 (25.5)	1.0	
The main purpose of cattle farming is for dairy production?	Yes	53/241 (22)	0.6 (0.4, 1.0)	0.10 ^a
	No	34/114 (29.8)	1.0	
The main purpose of cattle farming is for draught purposes?	Yes	19/42 (45.2)	2.9 (1.5, 5.7)	0.001 ^a
	No	68/313 (21.7)	1.0	
Cattle were tethered in the open air during the day?	Yes	19/81 (23.5)	0.9 (0.5, 1.6)	0.80
	No	68/274 (24.8)	1.0	
Cattle were free ranging during the day?	Yes	68/262 (26)	1.3 (0.7, 2.4)	0.28
	No	19/93 (20.4)	1.0	
Cattle were housed in a shed during the day?	Yes	1/33 (3)	0.08 (0.01, 0.6)	0.003 ^{a,b}
	No	86/322 (26.7)	1.0	
Cattle were tethered in the open at night?	Yes	22/73 (30.1)	1.44 (0.8, 2.5)	0.21 ^a
	No	65/282 (23)	1.0	
Cattle were free ranging at night?	Yes	3/11 (27.3)	1.1 (0.3, 4.4)	0.828
	No	84/344 (24.4)	1.0	
Cattle were housed in a shed at night?	Yes	64/281 (22.8)	0.6 (0.3, 1.1)	0.139 ^a
	No	23/74 (31.1)	1.0	

Table 5.7 (contd) Univariable analysis for “farmer-diagnosed FMD in Bhutan”.

Variable name	Answer	No. +ve/total (% FMD positive)	Odds ratio (95% CI)	P-value
Feeding and watering practices				
Are cattle fed commercial feed?	Yes	11/85 (12.9)	0.3 (0.19, 0.7)	0.004 ^a
	No	76/270 (28.1)	1.0	
Are cattle fed kitchen waste?	Yes	69/131 (52.7)	12.7 (7, 23)	0.000 ^a
	No	18/224 (8)	1.0	
Do cattle graze near the house?	Yes	70/268 (26.1)	1.4 (0.8, 2.6)	0.215 ^a
	No	17/87 (19.5)	1.0	
Do cattle graze in the forest?	Yes	59/174 (33.9)	2.8 (1.6, 4.6)	0.000 ^a
	No	28/181 (15.5)	1.0	
Drinking source is tap water.	Yes	42/201 (20.9)	0.6 (0.4, 1)	0.07 ^a
	No	45/154 (29.2)	1.0	
Drinking source is river or stream water.	Yes	45/164 (27.4)	1.3 (0.8, 2.1)	0.234 ^a
	No	42/191 (22)	1.0	
Drinking source is irrigation water.	Yes	11/29 (37.9)	2 (0.9, 4.4)	0.079 ^a
	No	76/326 (23.3)	1.0	
Drinking source is spring water.	Yes	43/157 (27.4)	1.3 (0.8, 2.1)	0.26
	No	44/198 (22.2)	1.0	
Cattle mix with other cattle herds at water source?	Yes	83/266 (31.2)	9.6 (3.4, 27.1)	0.000 ^{a b}
	No	4/89 (4.5)	1.0	
Mixing of cattle with 1-5 herds at watering points?	Yes	23/138 (16.7)	0.4 (0.2, 0.8)	0.006 ^a
	No	64/217 (29.5)	1.0	
Mixing of cattle with 6-10 herds at watering points?	Yes	30/77 (39)	2.4 (1.4, 4.2)	0.001 ^a
	No	57/278 (20.5)	1.0	
Mixing of cattle with >10 herds at the watering points?	Yes	29/49 (59.2)	6.2 (3.2, 11.7)	0.000 ^a
	No	58/306 (19)	1.0	

Table 5.7 (contd) Univariable analysis for “farmer-diagnosed FMD in Bhutan”.

Variable name	Answer	No. +ve/total (% FMD positive)	Odds ratio (95% CI)	P-value
Feeding and watering practices				
Animals are sent for grazing	Yes	86/308 (27.9)	17.8 (2.2, 131.2)	0.000 ^{a b}
	No	1/47 (2.1)	1.0	
Cattle graze on community land	Yes	20/64 (31.2)	1.5 (0.8, 2.7)	0.16 ^a
	No	67/291 (23)	1.0	
Cattle graze in paddy fields	Yes	30/76 (39.5)	2.5 (1.4, 4.3)	0.001 ^a
	No	57/279 (20.4)	1.0	
Cattle mix with other herds in the same village at grazing	Yes	85/264 (32.2)	21.1 (5, 87)	0.000 ^{a b}
	No	2/91 (2.2)	1.0	
Cattle mix with 1 to 5 herds within the same village at grazing	Yes	25/142 (17.6)	0.5 (0.3, 0.8)	0.014 ^a
	No	62/213 (29.1)	1.0	
Cattle mix with more than 6 herds within the same village at grazing	Yes	60/115 (52.2)	8.6 (5.0, 14.8)	<0.0001 ^a
	No	27/239 (11.3)	1.0	
Cattle mix with other herds from nearby villages	Yes	75/105 (71.4)	49.3 (24, 101.2)	<0.0001 ^a
	No	12/249 (4.8)	1.0	
Cattle mix with other herds from 1 to 5 villages	Yes	44/68 (64.7)	10.4 (5.7, 18.8)	<0.0001 ^a
	No	43/287 (15)	1.0	
Cattle mix with other herds from 6 to 10 villages	Yes	23/27 (85.2)	23 (7.9, 70.9)	<0.0001 ^{a b}
	No	64/328 (19.5)	1.0	
Cattle mix with other herds from more than 10 villages	Yes	9/11 (81.8)	15.3 (3.2, 72.5)	<0.0001 ^{a b}
	No	78/344 (22.7)	1.0	

Table 5.7 (contd) Univariable analysis of putative risk factors and response variable “farmer-diagnosed FMD in Bhutan”.

Variable name	Answer	No. +ve/total (% FMD positive)	Odds ratio (95% CI)	P-value
Other predictor variables				
Borrow bull	Yes	67/266 (25.2)	1.1 (0.6, 2.0)	0.606
	No	20/89 (22.5)	1.0	
Lend bull	Yes	63/260 (24.2)	0.9 (0.5, 1.6)	0.841
	No	24/95 (25.3)	1.0	
Do you buy cattle?	Yes	75/256 (29.3)	3 (1.5, 5.8)	0.001 ^a
	No	12/99 (12.1)	1.0	
Buy cattle from the same village	Yes	37/149 (24.8)	1.0 (0.63, 1.6)	0.90
	No	50/206 (24.3)	1.0	
Buy cattle from the same district	Yes	35/97 (36.1)	2.2 (1.3, 3.7)	0.002 ^a
	No	52/258 (20.2)	1.0	
Buy cattle from a different district	Yes	24/50 (48)	3.5 (1.9, 6.5)	0.000 ^a
	No	63/305 (20.7)	1.0	
Buy meat from the same village	Yes	4/33 (12.1)	0.3 (0.1, 1.1)	0.09 ^{a b}
	No	83/315 (26.3)	1.0	
Buy meat from other areas	Yes	78/264 (29.5)	3.4 (1.6, 7.3)	0.001 ^a
	No	9/84 (10.7)	1.0	
Buy dairy products	Yes	47/166 (28.3)	1.4 (0.8, 2.2)	0.173 ^a
	No	40/182 (22)	1.0	
Sell Cattle	Yes	40/171 (23.4)	0.8 (0.5, 1.4)	0.617
	No	47/183 (25.7)	1.0	
Cattle herd size				
Herd size	< 5	22/117 (18.8)	0.6 (0.3, 1.0)	0.08 [*]
	≥ 5	65/238 (27.3)	1.0	
Herd size	< 10	59/263 (22.4)	0.6 (0.4, 1.1)	0.125 [*]
	≥ 10	28/92 (30.4)	1.0	

^aVariables (with P-values <0.25) were offered to the multivariable logistic regression model. ^bResults of Fisher’s Exact test.

Table 5.8 Final logistic regression model

Description of variable	β^a	SE ^b	Wald ^c	Sig ^d	OR ^e (95% CI)
1. Cattle always housed in shed during day	-3.41	1.65	4.27	0.039	0.03 (0.001, 0.83)
2. Cattle always housed in shed at night	-1.23	0.53	5.38	0.020	0.29 (0.10, 0.82)
3. Cattle fed kitchen wastes	2.64	0.47	31.73	0.000	14.1 (5.6, 35.23)
4. Cattle sent for grazing in forest	1.12	0.45	6.07	0.014	3.1 (1.25, 7.46)
5. Cattle mixed with herds from other villages at grazing	3.67	0.48	57.93	0.000	39.28 (15.26, 101.08)
6. Cattle mixed with more than 6 herds in same village at grazing	1.66	0.45	13.57	0.000	5.30 (2.18, 12.89)
7. Interaction between “Cattle sent for grazing in forest” and “Cattle mixing with herds from other villages”	-2.38	0.90	7.02	0.008	0.092 (0.016, 0.537)
8. Constant	0.17	1.60	0.01	0.913	1.19

^a β =Estimate of the change in dependent variable attributed to a change of one unit in independent variable

^bSE=Standard error

^cWald=a test that a coefficient is zero based on the Wald statistic

^dSig=Significance for the Wald statistic

^eOR=Odds ratio

There was significant interaction between the variables “cattle mixing with herds from other villages at grazing” and “cattle sent for grazing in forest” and hence this was included in the final model (Table 5.8).

5.4 Discussion

Livestock farming in the study area was found to be extensively orientated. Farmers in the interior districts (Trongsa and Trashigang) reared mostly cattle whereas those residing in Sarpang and Chukha were found to rear multiple species including cattle, sheep, goats and pigs. Cattle were invariably sent to the forest and nearby community land for grazing purposes and this practice allowed cattle from nearby herds and villages to mix freely. In contrast, pigs were reared in enclosures and were rarely allowed to move freely in the villages. Similarly, sheep and goats were also found to be mostly stall-fed and therefore rarely allowed to move freely around the village. Foot-and-mouth disease, as diagnosed and reported by the farmers, was found to be endemic in the study area.

This study has identified six risk factors that, in combination, were found to be associated with the outcome variable “farmer-diagnosed FMD in Bhutan”. Of these, three were related to mixing of animals within or between villages at grazing areas and one was related to feeding of kitchen wastes to cattle. The two protective factors were related to whether or not cattle were always housed in sheds during the day and at night respectively.

This study has shown that mixing of animals usually occurs at grazing and watering points in Bhutan. It was demonstrated that there is large scale mixing of animals (mainly cattle) at the grazing areas in the FMD-endemic villages when compared to the non-endemic areas of Bhutan. Mixing of animals at water sources has been cited as an important risk factor for FMD in other endemic countries such as Thailand (Cleland *et al.*, 1996, Rojanasthien *et al.*, 2006), Cameroon (Bronsvort *et al.*, 2004a), Myanmar (Oo, 2010), and Cambodia (Sothyra, 2008). Although mixing at water sources was significantly associated with the risk of FMD in the univariable analyses, these factors were not included in the final model, perhaps due to their strong association with mixing at grazing. This is because grazing and watering rarely occur independently in Bhutan. Cattle sent for grazing in the forests or

community pastures also end up mixing with other herds at watering points. This observation can be further supported by the fact that approximately 89% (295/331) of the farmers who sent their cattle for grazing also replied that their animals watered with other herds at nearby springs or streams/rivers. Farmers who allowed their cattle to mix with herds from other villages were 39.2 times (95% CI: 15.2, 101.08; $P < 0.0001$) more likely to report FMD in their herds than those who didn't. Cleland *et al.* (1996) also reported that the risk of being in a high FMD frequency group was increased to 2.4 times for each additional village with which livestock mixed at water sources in Thailand. It is likely that close contact between animals during grazing and watering leads to the spread of FMD both within and between villages in Bhutan. Movement of infected animals is the most important factor in the spread of FMD in endemic areas (Rweyemamu *et al.*, 2008b) and infected animals can introduce disease to disease-free herds/flocks by both direct or indirect contact arising from mixing (Christley *et al.*, 2005). This indicates that spread from nearby infected herds and villages is an important source of FMD outbreaks for other non-infected herds. Therefore, quarantine of early cases in the first affected herd or village could reduce the spread to other herds and villages. This calls for enhanced surveillance in the villages whereby outbreaks of FMD are reported promptly to the nearest livestock office. Therefore, there is a need to create more awareness among the farmers in the FMD-endemic villages about the need to adopt simple biosecurity procedures such as disinfection of the sheds, confinement of the animals for 7-10 days, and feeding of kitchen wastes only after appropriate heat treatment (cooking).

Farmers in Bhutan are known to feed their livestock, including pigs and cattle, with kitchen wastes as supplementary feed. This practice is still prevalent, particularly amongst poor farmers who cannot afford to purchase commercial feed or develop improved pastures for their cattle. The majority of Bhutanese consume meat (especially pork and beef) and

therefore it is likely that in some instances kitchen wastes containing FMDV-contaminated materials from meat-washings or offal could be fed to the animals. The farmers also eat sun-dried or air-dried beef and pork and there is the possibility of these foods also entering the food chain of cattle. Animals, especially pigs, can be infected through the ingestion of contaminated uncooked swill (Alexandersen *et al.*, 2003b). The outbreaks in South Africa in 2000 and the UK in 2001 have been linked to the feeding of untreated waste food to pigs (Knowles *et al.*, 2001). The source for the Japan 2000 epidemic in Miyazaki Prefecture has also been linked to feeding of contaminated fodder (wheat straw) to cattle (Knowles *et al.*, 2001, Sugiura *et al.*, 2001).

It is plausible that the remains of infected meat can enter the food chain of cattle and lead to outbreaks. It has been well established that significantly higher doses of virus are required to result in infection through ingestion of FMDV-contaminated materials as compared to inhalation (Donaldson, 1987). However, abrasions in the oral cavity as a result of injury caused by sharp objects may facilitate infection by contaminated waste food (Alexandersen *et al.*, 2003b).

It is unlikely that pigs had a role to play in infecting cattle after acquiring infection through ingestion of kitchen wastes. This is because of the very low population (2.3% of all animals) distributed in only 13% of the households. Univariable analysis also showed that the presence of pigs in the herd was not significantly associated ($P=0.17$, Table 5.7) with outbreaks of FMD. None of the farmers who diagnosed FMD in their herds reported seeing clinical signs of FMD in the pigs. However, further studies are needed to confirm this observation.

There is a need to create awareness among the livestock owners, especially in the FMD-endemic villages, to adopt better management and feeding practices in order to reduce the chances of FMDV-contaminated materials getting into the food chain of cattle and other

species. The DOL's strategy of reducing the number of unproductive cattle by exchanging them with exotic breeds, at the ratio of 3-4 unproductive animals to one improved breed, needs to be implemented in these areas so that farmers will start adopting improved management and feeding practices for the introduced improved breeds as compared to the local breeds. With a lower herd size resulting from the use of improved cattle, farmers can provide better feed to their animals. Since the FMD virus can be inactivated at 56°C in 30 minutes (Donaldson, 1987), farmers should also be encouraged to cook kitchen waste before feeding it to animals in order to reduce the risk of disease transmission.

Risk factor studies performed elsewhere have identified owning buffalo (Bronsvort *et al.*, 2004a), owning sheep and goats (Megersa *et al.*, 2008), purchasing of livestock at markets (Lindholm *et al.*, 2007), cattle herd density (Perez *et al.*, 2004), and a close proximity to slaughter facilities (Rojanasthien *et al.*, 2006, Lindholm *et al.*, 2007) as potential risk factors associated with the transmission of FMDV. These risk factors were not found to be important or relevant in this study. This could be due to differences in livestock husbandry practices, socio-economic conditions, people's dietary preferences or geo-physical conditions between the current study area and other studies.

For instance, in this study, buffalo, sheep and goat represented only 0.2%, 2%, and 6.6% of the total livestock population in the study area respectively. Therefore, based on their relatively small numbers, when compared with cattle, these species probably have a relatively insignificant role in the dispersal of FMD in Bhutan. This observation can be further supported by the fact that small ruminants and pigs were found to be rarely affected with FMD in Bhutan (Chapter 3).

In Bhutan, there are no organized livestock markets in the villages and towns unlike in other South Asian countries. Therefore, there are no large scale gatherings of livestock for

the purpose of buying or selling animals. Therefore currently livestock markets do not seem to have any role as potential risk factors for the spread of FMD in Bhutan.

The model identified two protective factors associated with the occurrence of outbreaks of FMD in Bhutan. This is likely to be linked to a lowering of the probability of contact with other animals in the village both during the day and night time. Housing cattle in sheds reduces the opportunity for the animals to mix with other animals within villages and therefore reduces the potential for disease transmission among the animals. The reasons for the differences in the protection levels provided by night-housing and day-housing cannot be explained but perhaps could be related to the patterns of mixing.

The logistic regression model “Farmer-diagnosed FMD in Bhutan” was assessed for overall model fit and the variables in the final model were checked for interactions as described earlier. Two variables “cattle mixing with herds from other villages at grazing” and “cattle sent for grazing in forest” had significant interactions ($P=0.008$). This could be due to the fact that cattle sent for grazing in forest could have also mixed with herds from other villages. The survey showed that 32% (93/287) of the farmers who sent their cattle for grazing in the forest also allowed their animals to mix with herds from other nearby villages. Usually animals from nearby villages share common grazing and watering areas in Bhutan, due to a shortage of feed.

Since this study was confined to villages located near the road points, extrapolation of results from this study to the entire country has to be done with caution. However, the livestock production and management system is subsistence orientated and extensively managed, even in remote villages, and therefore the situation in other parts of Bhutan is similar to the study area. Therefore, findings from this study are, to a certain extent, representative of the situation in other areas of Bhutan. As with all questionnaire-based studies, some of the disadvantages could include the influence of recall bias, interviewer

bias and failure to validate the questionnaire responses by repeating the questionnaire survey among the same respondents (Bronsvooort *et al.*, 2003). Recall bias is less likely to occur as FMD produces characteristic clinical signs, especially in cattle, although the disease could be easily missed in small ruminants (Davies, 2002). Cattle constituted the majority of the FMD-susceptible species in the study area and therefore the disease could be easily diagnosed by the farmers and field veterinarians. This was confirmed by the finding that the majority (93%) of the respondents could recognise the clinical signs or lesions of FMD when a picture of FMD was shown. Similarly, 87% of the respondents could recall some of the clinical signs and lesions of FMD.

The logistic regression model has produced interesting insights into the “local” epidemiology and the association of potential risk factors for FMD in Bhutan. The role of mixing of animals at grazing and watering areas, both within and between villages, as risk factors for FMD has been highlighted. The risk was found to increase exponentially with an increase in the number of herds from the same village and herds from other villages with which animals mix.

In conclusion the study has identified and quantified six risk factors that could, in combination, best explain the variation in FMD occurrence in Bhutan.

The study has identified the need for active surveillance (clinical and serological) to rapidly detect infection in the population so that effective disease control measures can be put into place before there is widespread transmission of disease. There is a need to validate the responses of the farmers in order to have confidence in the routine use of questionnaire surveys for active surveillance of FMD in Bhutan. The next chapter, on seroprevalence, provides data to validate the responses obtained from the current chapter.

CHAPTER SIX

The seroprevalence of foot-and-mouth disease in the sedentary livestock herds in four districts in Bhutan

This Chapter has been published in a peer-reviewed journal:

Dukpa, K., Robertson, I.D. and Ellis, T.M. (2011) The seroprevalence of foot-and-mouth disease in the sedentary livestock herds in four districts in Bhutan. *Preventive Veterinary Medicine*, **100 (3-4)**, 231-236.

6.1 Introduction

As outlined in Chapter 1, Bhutan has a passive disease surveillance system based mainly on disease reporting by the livestock owners and veterinary field staff. Due to various reasons including the remoteness of villages, poor communication facilities, lack of incentives for disease reporting both for the farmer and the extension agent, the endemic nature of the disease, and low mortality, many outbreaks could go unreported as is inherent in a passive surveillance system (McLeod, 2003, Sumption *et al.*, 2008, Oo, 2010). Consequently not all outbreaks are recorded in the VIS and control activities will not be based on correct disease epidemiology.

The questionnaire interview with the farmers (Chapter 5) showed variable prevalences to FMD in herds and villages. The retrospective study based on 13 years of passive surveillance (1996-2008) showed that more outbreaks of FMD occurred in sub-

districts/districts bordering India (Chapter 3) than in the interior sub-districts. It was also found that cattle were the main species affected while other species, such as small ruminants and pigs, were rarely affected. However this information has not been validated with epidemiologically designed serological surveys. Therefore, there was a need to undertake seroprevalence studies to validate the above findings, as well as to establish the prevalence of FMD in various agro-ecological zones.

Although FMD is endemic in Bhutan, no active sero-surveillance has previously been undertaken. A correct estimate of the prevalence of a disease is crucial in order to design and undertake epidemiological studies, particularly for estimating sample size and working out the precision of a study. The estimated prevalence can also be used in future studies to monitor the success of a control programme (Thrusfield, 2005).

Animals infected with FMDV produce antibodies to both structural as well as non-structural proteins (Robiolo *et al.*, 2006). The circulating antibodies can be detected as early as three to five days after the appearance of the first clinical signs and are known to peak at around 14 days post infection (Alexandersen *et al.*, 2003b). Animals vaccinated against FMD using purified vaccines are expected to produce antibodies to viral structural proteins only (OIE, 2008). Serological surveys have been used in many countries such as Lao PDR (Blacksell *et al.*, 2008), Cameroon (Bronsvort *et al.*, 2006), Taiwan (Chung and Liao, 2003, Chung *et al.*, 2003, Chen *et al.*, 2008), Thailand (Doughty *et al.*, 1995), Uganda (Balinda *et al.*, 2009, Mwiine *et al.*, 2010), Egypt (Maanen *et al.*, 2010) and Kazakhstan (Lundervold *et al.*, 2004) to determine the prevalence of FMD, as well as to assess the control programmes. Animal health surveillance is an essential tool to monitor disease trends and to facilitate the control of disease or infection (OIE, 2009a). Serological testing is a suitable tool for FMD surveys (OIE, 2009a) and in areas where animals have

been routinely vaccinated, NSP tests should be used to monitor for FMDV infection or virus circulation (OIE, 2009a).

For the purpose of this chapter, sedentary herds are defined as those herds that do not take part in seasonal migration or move long distances, except for the daily movements for grazing and watering purposes in and around their usual place of residence.

This study was undertaken with the following objectives.

- To establish the seroprevalence/distribution of FMD in sedentary herds in different agro-ecological zones of Bhutan
- To validate the findings of the passive surveillance system and farmer-diagnosed FMD for the 5-years preceding this survey.

6.2 Materials and methods

6.2.1 Study area

The seroprevalence study was undertaken in the four districts of Chukha, Sarpang, Trongsa and Trashigang as previously described (Chapter 5).

6.2.2 Sampling strategy

Since diseases in animal populations are known to cluster at a herd or village level, a low proportion of villages may be affected whereas a large proportion of animals within that village can be diseased (Cameron and Baldock, 1998). Therefore multi-stage sampling was undertaken in order to account for this clustering effect so that the results of the survey could be extrapolated to the target population. A stratified, multi-stage, probability proportional to size (PPS) sampling method was used to sample the villages and the animals. All villages in the selected districts that were within approximately half-an-hour walking distance from the nearest road point were listed in the sampling frame. The latest village-level livestock census was obtained from the respective district livestock offices

and a sampling frame constructed. Villages with a higher cattle population were more likely to be selected than those with fewer animals. Villages were considered as the primary sampling units and animals as the secondary sampling units.

Once a village was selected, a list of households owning livestock was generated and then the households were selected by simple random sampling. Around four to five households per village were randomly selected and from each selected household, four to five locally reared animals were selected using a simple random sampling method. Since not all households and villages contained pigs, targeted sampling was undertaken for this species. Ageing of the animals was done based on the knowledge of the farmer and all sampled animal's age were recorded.

The first sampling was undertaken between March and May 2009 following approval by the Murdoch University Animal Ethics Committee. During the first sampling, blood was collected from cattle, sheep and goats only as it was logistically difficult to sample pigs along with the former species. Pigs were sampled from September to December 2009.

6.2.3 Sample size

With a total of 800 villages and an average of 200 FMD-susceptible animals per village in the study area and a cost ratio of 100:1 of villages against animals, the Survey Tool box version 1.0 beta (Cameron, 1999) gave a two stage sample size of 77 villages and 20 animals per village. A total of 1540 animals needed to be sampled to give a 95% confidence level of estimating a seroprevalence of 20% at the individual animal level with a precision of $\pm 5\%$. Since no such studies had been done in the country before, an assumed within-village variance of 0.20 and a between-village variance of 0.05 were used. Similar values had been used elsewhere in South Asia to determine sample size (Cameron, 1999).

The total sample size was finalized at 1600 to make up for the potential failure of obtaining sera from some animals or to account for haemolysed sera which would be unfit for testing. After stratification by districts, the final sample size was 20 villages per district and at least 20 animals per village in each district to result in a total sample of 400 animals per district. When sheep and goats were present in the villages, cattle accounted for 80% of the sample size and the remaining 20% came from the small ruminants and pigs. This proportion was based on the fact that cattle, small ruminants and pigs constituted approximately 72.6%, 9.2% and 6.1% of the total FMD-susceptible population, respectively, in Bhutan in 2007 (Anonymous, 2009a). In the actual survey 1909 animals were sampled as a result of excellent cooperation of the livestock farmers and the field staff engaged in sampling.

6.2.4 Sample collection

The purpose of the survey was explained to the farmers and their consent was obtained before the survey was undertaken. Volumes of blood collected ranged from 1 to 7 ml per animal, depending on the species, using a 20 gauge needle and a plain vacuum-containing tube. Blood was allowed to stand for 12 – 24 hours (depending on the ambient temperature) to clot before serum was decanted into a vial. Those samples which failed to clot (due to a cold ambient temperature) were centrifuged at 2500 rpm (revolutions per minute) for 5 minutes to separate the sera. All sera were coded, sealed and preserved at -20°C in a freezer until testing.

6.2.5 Laboratory tests

As the study population had been vaccinated against FMD (trivalent vaccine containing serotypes O, A, and Asia 1 from India) in the years preceding the time of survey, FMD non-structural protein (NSP) 3ABC ELISA tests that could differentiate antibodies induced

by natural infection from that induced by vaccination were used (Kitching, 2002a, OIE, 2008). A commercial NSP kit, PrioCHECK[®] FMDV NS, from Prionics AG (Switzerland), was used for the seroprevalence study since the kit has been reported to have a high sensitivity and specificity compared with other commercially available NSP kits (Brocchi *et al.*, 2006, Engel *et al.*, 2008) and can be used in all domestic animals including pigs. The test was used according to the manufacturer's instructions and as previously reported (Sørensen *et al.*, 1998, Sørensen *et al.*, 2005). All laboratory tests were conducted at the National Centre for Animal Health in Bhutan.

A percentage inhibition (PI) of <50% was considered negative and a PI of ≥50% was classed as positive to indicate the presence of anti-NSP antibodies in the tested animal. All positive sera were re-tested using the same kit to reduce false positives.

6.2.6 Data analysis

Data were managed and analysed as described in previous Chapters. The seroprevalence for each district was calculated by dividing the number of ELISA positive samples from that district by the total number of samples tested from that district. A herd or a village was considered as NSP-positive if at least one animal in the herd or village was seropositive on the NSP test (Bronsvooort *et al.*, 2006). The 95% confidence interval for the test prevalence was calculated based on the exact binomial method (Ross, 2003). The true prevalence was calculated using an on-line epidemiological calculator (<http://epitools.ausvet.com.au>) developed by Sergeant (2009) based on methods described by Rogan and Gladen (1978). For data that were not normally distributed, even after log transformation, non-parametric tests such as Mann-Whitney U test and Kruskal-Wallis

tests were used to detect significant differences in the proportional seropositivity between districts and species. Pearson’s Chi-square tests were used for the categorical data.

For the purpose of this chapter, a ‘local’ breed was defined as those animals with no breed specification and included all indigenous animals and animals with no clear breed specification or description. ‘Improved’ breed was defined as those animals which are crossed with established exotic breeds such as Jerseys or Brown Swiss and which have some phenotypic characteristics of these exotic breeds.

6.3 Results

6.3.1 Sampling profile

Blood was collected from a total of 1909 animals including cattle, goats, pigs and sheep originating from 485 herds located in 106 villages in the four districts (Table 6.1). The samples originated from villages located at altitudes between 232 and 3022 metres above sea level (m.a.s.l.). Cattle (79.4%) constituted the bulk of the sampled animals followed by pigs (13.6%), goats (5.7%) and sheep (1.3%). A summary of the species-wise sera collected from each of the districts is displayed in Table 6.2.

Table 6.1 Summary of sampling done for the seroprevalence study

Districts	No. of sub-districts sampled	No. of villages sampled	No. of herds sampled	Total number of sera collected
Chukha	7	31	134	535
Sarpang	9	26	107	492
Trashigang	9	26	110	434
Trongsa	4	23	134	448
Total	29	106	485	1909

Table 6.2 Species-wise distribution of samples (%)

Districts	Cattle	Goats	Pigs	Sheep
Chukha	365 (68.2)	41 (7.7)	124 (23.2)	5 (0.9)
Sarpang	356 (72.4)	58 (11.8)	62 (12.5)	16 (3.3)
Trashigang	400 (92.2)	8 (1.8)	26 (6.0)	0 (0.0)
Trongsa	395 (88.2)	1 (0.2)	48 (10.7)	4 (0.9)
Total	1516 (79.4)	108 (5.7)	260 (13.6)	25 (1.3)

The majority (69%, n=1322) of the sera collected were from females. This trend was true for all species (Table 6.3) except for pigs where more samples were collected from males (65.8%) than females (34.2%).

Table 6.3 Composition of samples by gender and breed (%)

Species	Gender		Breed	
	Male	Female	Local	Improved
Bovine*	368 (24.3)	1147 (75.7)	830 (54.7)	686 (45.3)
Caprine	39 (36.1)	69 (63.9)	108 (100)	0 (0.0)
Ovine	8 (32.0)	17 (68.0)	25 (100)	0 (0.0)
Porcine	171 (65.8)	89 (34.2)	108 (41.5)	152 (58.5)
Total	586 (30.7)	1322 (69.3)	1071 (56.0)	838 (44.0)

*Note: For one bovine serum from Sarpang district (S394), no gender was recorded.

More than half of the samples (56%) were collected from local breeds as compared to improved/imported breeds.

6.3.2 Seroprevalence at the animal level

Using the sensitivity and specificity values of 97.2% and 99.5%, respectively, for the PrioCHECK kit (Sørensen *et al.*, 2005, Brocchi *et al.*, 2006, Kittelberger *et al.*, 2008), the true prevalence at the animal-level for all species was 15% (95% CI: 13.5, 16.7). Cattle had the highest true prevalence followed by goats, sheep and pigs (Table 6.4).

Sarpang had the highest overall seroprevalence in all species, followed by Chukha, Trongsa and Trashigang (Table 6.4). The prevalence of NSP-antibodies varied significantly between districts for cattle ($P<0.0001$, $\chi^2=66.3$, $df=3$) and between species ($P<0.0001$, $\chi^2=40.9$, $df=3$) with the highest test/apparent prevalence being recorded in cattle (17.5%, 265/1516) followed by goats (12%, 13/108), sheep (12%, 3/25) and pigs (2.3%, 6/260) (Table 6.4).

Table 6.4 Animal-level seroprevalence (test prevalence) of FMD

Districts	Test Prevalence (95% CI)			
	Cattle	Goats	Sheep	Pigs
Chukha	16.2 (12.5, 20.3)	14.6 (5.6, 29.2)	0.0 (0.0, 52.2)	4.0 (1.3, 9.2)
Sarpang	31.2 (26.4, 36.3)	12.0 (5.0, 23.3)	18.8 (4.0, 45.6)	1.6 (0.0, 8.7)
Trashigang	9.8 (7.0, 13.1)	0.0 (0.0, 36.9)	NSA	0.0 (0.0, 13.2)
Trongsa	14.2 (10.9, 18.0)	0.0 (0.0, 97.5)	0.0 (0.0, 60.2)	0.0 (0.0, 7.4)
Overall	17.5 (15.6, 19.5)	12.0 (6.6, 19.7)	12.0 (2.5, 31.2)	2.3 (0.9, 5.0)

NSA = No sheep available for sampling

Only goats originating from Chukha or Sarpang districts were positive (Table 6.4).

Similarly, three sheep from Sarpang were positive for FMD. Only pigs from Sarpang and

Chukha tested positive to the NSP test.

6.3.3 Seroprevalence at herd- and village-level

The seroprevalence of FMD at the herd and village-level were 37.7% (95% CI: 33.4, 42.2)

and 66.9% (95% CI: 57.2, 75.8), respectively (Table 6.5).

Table 6.5 Seroprevalence (all species) of FMD at herd- and village-level

Districts	No. of villages (positive/ tested)	Village-level seroprevalence (95% CI)	No. of herds (positive/ tested)	Herd-level seroprevalence (95% CI)
Chukha	22/31	70.9 (52.0, 85.8)	44/134	32.8 (25.0, 41.5)
Sarpang	20/26	76.9 (56.4, 91.0)	71/107	66.4 (56.6, 75.2)
Trashigang	13/26	50.0 (29.9, 70.1)	26/110	23.6 (16.1, 32.7)
Trongsa	16/23	69.6 (47.1, 86.8)	42/134	31.3 (23.6, 39.9)
Total	71/106	66.9 (57.2, 75.8)	183/485	37.7 (33.4, 42.2)

The herd-level seroprevalence of FMD varied significantly between districts, with Sarpang (66.4%) having a higher seroprevalence than the other districts ($P < 0.01$, $\chi^2 = 50.3$, $df = 3$).

The distribution of seropositive animals in the districts is given in Table 6.6.

Table 6.6 Distribution of number of seropositive animals in the seropositive villages

Districts	No. of villages (positive/ tested)	No. of villages with one seropositive animal in each village	No. of villages with 2-4 seropositive animals in each village	No. of villages with 5 and more seropositive animals in each village
Chukha	22/31	4	13	5
Sarpang	20/26	3	5	12
Trashigang	13/26	7	4	2
Trongsa	16/23	3	7	6
Total	71/106	17	29	25

Sarpang (n=12) and Trashigang (n=2) had the highest and lowest number of villages that had five or more seropositive animals, respectively.

6.3.4 Age-stratified seroprevalence

The median age of seropositive cattle was significantly higher than the seronegative group in all districts (Table 6.7, $P < 0.0001$, $z = -10.2$). The seroprevalence of FMD for cattle increased with the age and this increase was apparent in all districts (Figure 6.1).

Figure 6.1 Age-stratified seroprevalence of FMD in cattle from four districts

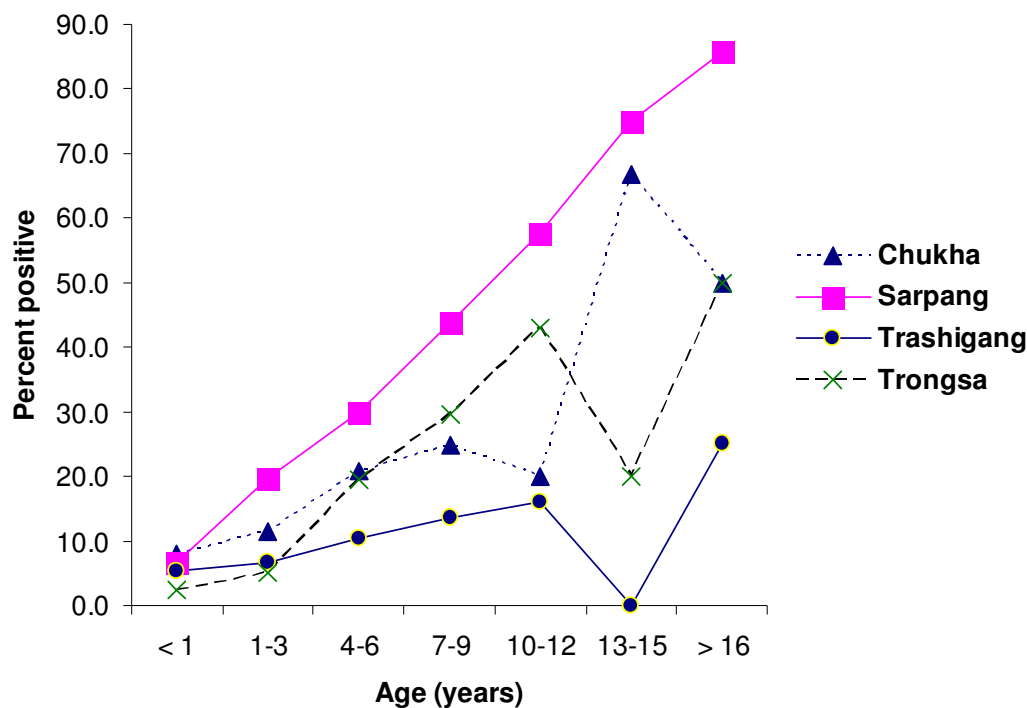


Table 6.7 Difference in age (in years) between seropositive and seronegative animals

Species	Median age of seronegative group (range)	Median age of seropositive group (range)	P-values (Mann-Whitney U tests)
Cattle	4.0 (0.3 – 25.0)	6.0 (0.5 – 20.0)	P<0.0001, z = -10.2
Goats	2.0 (0.2 – 13.0)	2.0 (0.2 – 6.0)	P=0.683, z = -0.4
Sheep	2.0 (0.2 – 8.0)	3.0 (2.0 – 9.0)	P=0.177, z = -1.4
Pigs	0.6 (0.2 – 3.0)	1.5 (0.2 – 3.0)	P=0.068, z = -1.8
All species	3.0 (0.2 – 25.0)	6.0 (0.2 – 20.0)	P<0.0001, z = -10.9

6.3.5 Influence of breed on seropositivity

Local breeds of cattle (20.7% prevalence; 95% CI: 18, 23.5) were 1.67 times more likely (P<0.0001, $\chi^2=13.37$, df=1) to be seropositive than were improved breeds (13.6%, 95% CI: 11, 16.1). No analysis was done for goats and sheep and pigs because these species were only represented by local breeds.

6.3.6 Influence of previous infection on seropositivity

The previous infection status of the sampled herds and villages, based on the farmers' responses to the questionnaire survey (Chapter 5), was compared with the serological results. Farmers were asked whether or not they had seen an outbreak of FMD in their herd within the 5-years preceding the date of this serological survey (Chapter 5).

Herds with known outbreaks of FMD within the last 5-years had significantly ($P < 0.001$, $\chi^2 = 93.4$, $df = 1$) higher prevalence of anti-NSP antibodies (33.9%; 95% CI: 29, 38.8; $n = 123$) than those with no history of FMD outbreaks (12.3%; 95% CI: 10.5, 14.1; $n = 158$). The odds of being seropositive was 3.6 times (95% CI: 2.7, 4.8) more likely in herds with known outbreaks than those originating from herds with no history of FMD.

The outbreak data available in the VIS (Anonymous, 2009b), the responses of the farmers (Chapter 5) on whether they had had an outbreak of FMD in their herds in the 5-years preceding the survey, and the results of the village-level NSP seroprevalence were compared for each district (Table 6.8). The passive surveillance system (Anonymous, 2009b) showed only 19 of 106 villages as having an outbreak of FMD whereas the questionnaire and serological surveys identified 49 and 71 villages, respectively, as having an outbreak during the 5-years preceding the study. There were significant differences ($P < 0.0001$, $\chi^2 = 52.24$, $df = 2$) in the sensitivities of these three methods.

Of the 16 villages having goats that had recorded an outbreak of FMD, six had at least one seropositive animal. Of the 16 villages having goats that didn't record an outbreak of FMD, four had seropositive animals. Of the four villages having sheep that had recorded an outbreak of FMD, one village had seropositives. Of the five villages having sheep that didn't record outbreaks of FMD, one had a seropositive animal.

Table 6.8 Comparison of the three methods of disease surveillance for diagnosis of foot-and-mouth disease at village-level in Bhutan

Districts	No. of villages in the seroprevalence study	No. of villages (%) reporting an outbreak of FMD between 2004 and 2008 as per official records (VIS)*	No. of villages (%) reporting an outbreak of FMD between 2004 and 2008 as per questionnaire survey [#]	No. of villages (%) with at least one seropositive animal to NSP test ⁺
Chukha	31	5/31 (16.1)	14/31 (45.2)	22/31 (70.9)
Sarpang	26	9/26 (34.6)	11/26 (42.3)	20/26 (76.9)
Trashigang	26	3/26 (11.5)	8/26 (30.7)	13/26 (50.0)
Trongsa	23	2/23 (8.7)	16/23 (69.6)	16/23 (69.6)
Total	106	19 (17.9%)	49 (46.2%)	71 (66.9%)

*Information from passive surveillance system; [#]Information based on questionnaire survey of the farmers; ⁺seroprevalence study

6.3.7 Influence of altitude on seropositivity

The seroprevalence (19.3%, 95% CI: 16.6, 22.3) in the sub-tropical zone (<1200 m.a.s.l) was significantly ($P < 0.0001$, $\chi^2 = 21.96$, $df = 2$) higher than the warm temperate zone (1201-2500 m.a.s.l; prevalence=12.9%, 95% CI: 10.9, 15.1) or the cool temperate zone (>2500 m.a.s.l; prevalence=6.3%, 95% CI: 2.8, 12.2).

6.3.8 Influence of sharing borders with India on seropositivity

Those sub-districts that shared a border with India had a significantly ($P = 0.03$, $\chi^2 = 4.72$, $df = 1$) higher seroprevalence (17.6%, 95% CI: 14.7, 20.9) than those that didn't (13.8%, 95% CI: 12, 15.8). These sub-districts were 1.3 times (95% CI: 1.03, 1.73) more likely to have seropositive animals than those not sharing a border with India.

6.4 Discussion

The study demonstrated that FMD is endemic in Bhutan with sub-districts and districts bordering India having higher seroprevalence than the interior ones. Similar observations were also made from the country's passive surveillance system (Dukpa *et al.*, 2011).

Sarpang and Chukha districts share their borders with the Indian states of Assam and West Bengal, respectively, where FMD is endemic (Sarma and Sutopa, 2003, Bhattacharya *et al.*, 2005) and there is unrestricted movement and mixing of animals at the border areas (Dukpa and Pem, 2007a). The high seroprevalences in Sarpang and Chukha districts could be attributed to the outbreaks that had occurred in the sampled villages, some of which occurred as recently as 2007 and 2008. Anti-NSP antibodies, especially the anti-3ABC antibodies, can be detected in infected animals for up to 665 days (Moonen *et al.*, 2004b) following infection. Therefore, it is likely that the infected animals in these districts still had detectable levels of circulating anti-NSP antibodies derived from previous infection. Sarpang district also had significantly higher species-level seroprevalence than the other three districts for cattle (31.2%) and sheep (18.8%). The last officially recorded outbreaks of FMD (Anonymous, 2009b) in Chukha, Sarpang, Trashigang, and Trongsa were in 2008, 2007, 2004 and 2006, respectively.

Sarpang and Chukha districts also had significantly higher outbreaks of FMD than other districts between the years 1996 and 2008 as per the passive surveillance system (Chapter 3). In Sarpang, 11 of the 18 villages sampled had an outbreak of FMD in 2007 as per the questionnaire survey (Table 5.4; Chapter 5). In Chukha, 14 of the 22 villages had an outbreak of FMD, some of which occurred in 2008. Farmers in Trashigang (Table 5.4, Chapter 5) reported FMD in eight of the 20 villages sampled and some of these outbreaks occurred in 2006. Sixteen of 19 villages sampled in Trongsa had recorded an outbreak of FMD within 5-years preceding this survey (Table 5.4, Chapter 5), some of which occurred in 2005 and 2006.

The low seroprevalences in two districts, in particular Trashigang district, could be attributed to the relatively low incidence of outbreaks (Anonymous, 2009b). Alternatively the NSP-antibodies in previously infected animals could have waned, since the last

reported outbreak of FMD in these districts was in 2006 (three years preceding this survey).

Cattle had the highest seroprevalence of the species sampled, followed by small ruminants with pigs having the lowest. In a structured seroprevalence study of village cattle and pigs in Lao PDR, using LPBE, Blacksell *et al.* (2008) also reported a significantly higher seroprevalence in cattle (16.7% for O serotype) as compared to pigs (0% to 0.04% only). Seropositive goats and sheep all originated from the districts of Sarpang and Chukha while none came from Trongsa or Trashigang. This again reflects the relatively higher incidence of outbreaks of FMD in the districts bordering India than in the interior districts. Given the extensive farming systems where multiple species, including cattle and small ruminants, are reared together (Chapter 5), small ruminants would also be exposed to FMDV infection. In this study, only a few villages that had sheep or goats had seropositives in these species. Although the seroprevalence in goats and sheep, at the animal-level, was relatively high, this could have been overestimated due to the small sample size. For instance, only 25 sheep were sampled and most of these originated from FMD-endemic villages. However, given the low population and scattered distribution, small ruminants do not seem to play an important role in the dispersal of FMD in Bhutan even though sheep can remain as carriers for up to 9 months (Burrows, 1968b, Pay, 1988); and goats for up to 4 months (Alexandersen *et al.*, 2002). The seroprevalence in pigs was the lowest and none of the pigs from the interior districts tested positive. Given their low numbers (6.1% of the total FMD-susceptible population, (Anonymous, 2009a)), management system adopted for rearing of pigs (Chapter 5), and the findings of this study, pigs do not appear to play a significant role in the dispersal of FMD in Bhutan as has been reported in previous studies (Dukpa *et al.*, 2011) as well as studies in other countries with similar management systems (Chamnanpood *et al.*, 1995). Given the relatively high seroprevalence in cattle and the fact

that they can act as carriers for up to two and half years (Hedger, 1968), this species should be considered as the priority species for future disease prevention and control programmes in terms of vaccination, movement control and surveillance. However, the small ruminants, especially unvaccinated goats, can be used as tracers in areas where cattle are routinely vaccinated.

Seropositive cattle were found to be significantly older than seronegative cattle. The prevalence increased in a linear manner with increasing age. The higher seropositivity in older cattle could be due to the increased potential for exposure of adult animals to FMDV at grazing and watering places as compared to younger animals. Young calves are mostly kept tethered at home while the adults are sent for grazing in the forest. In an abattoir-based serological survey of FMD in Lao PDR, using the liquid phase blocking ELISA, Blacksell *et al.* (2008) made similar observations. Similar findings have also been cited in a serological survey of FMD in indigenous cattle of Southern Ethiopia (Megersa *et al.*, 2008), using a 3ABC NSP ELISA, where the odds of seropositivity to NSP-antibodies were 2.8 and 2.3 times higher in adults (>4 years) and maturing animals (3-4 years) compared to young animals (<3 years). In a retrospective serological survey of FMD in the African buffalo (*Syncerus caffer*) using the CEDI test kit (now known as PrioCHECK[®] FMDV NS), Bronsvort *et al.* (2008) found a positive correlation between age and seropositivity to NSP antibodies. Similar observations were also made in Brazil (Picão-Gonçalves *et al.*, 2003) during a random serosurveillance study using NSP tests to prove freedom from infection.

More cattle of 'local' breeds were seropositive than 'improved' breeds in all districts. This could be due to differences in the management of the different breeds based on their economic value. The local breeds, because of their relatively low economic value, are not intensively managed by the farmers and are mostly kept tethered in fields or let loose in the

forest for grazing where they potentially mix with herds from the same or other villages. In contrast the improved breeds are mostly housed in sheds and are rarely sent for grazing in the forest (personal observation). Similar observations of differing seroprevalences attributable to farm management system for exotic and local cattle breeds have also been made in Uganda (Balinda *et al.*, 2009).

Animals originating from herds with known outbreaks of FMD (farmer-diagnosed) within the last 5-years were more likely to be seropositive than were those originating from herds with no history of FMD. This shows the usefulness of using NSP serology for routine surveys of FMD in Bhutan. Conversely, this also shows that participatory epidemiology, using structured questionnaires, can be an equally cost effective means of disease surveillance for FMD as has been shown in other countries (Oo, 2010). For an overall effective disease surveillance, both participatory (questionnaire) and serological surveys should be used together to complement each other (OIE, 2009a) in any future studies.

So far, surveillance for FMD in Bhutan has been based only on passive surveillance methods such as reporting of outbreaks by livestock farmers and extension workers to the national veterinary services. The pitfall of depending on this system alone is clearly outlined in this study where many outbreaks of FMD that occurred in the study area were not reflected in the national database. Similar observations were made in Myanmar (an FMD-endemic country) where only 20.7% of the farmers that had recorded FMD in their herds actually reported the outbreak to the local government veterinary office (Oo, 2010). One of the drawbacks of a passive surveillance system is the underreporting of cases (McLeod, 2003, Sumption *et al.*, 2008, Oo, 2010) and this has been confirmed in the present study. Serological surveys were useful in detecting infection in populations and villages that, according to the official records, were considered not to have had an outbreak of FMD.

Since the study was conducted in villages and herds that were located within approximately half-an-hour walking distance from the nearest road, caution needs to be taken in extrapolating the results to other villages, herds or districts in Bhutan. Cattle, sheep and goats were all randomly selected. Therefore sampling error in these species is unlikely. For pigs, however, convenience sampling was done as per the availability of pigs in the villages and therefore there is likely occurrence of sampling bias. Since the herds' or villages' infection statuses were based on the presence of at least one seropositive animal, given the test's imperfect sensitivity and specificity, misclassification is likely, at least at the herd-level. However, this is less likely to occur at the village level as only 24% (17/71) of the seropositive villages had only one seropositive animal. All NSP positives were retested to reduce the number of false positives and thereby reduce the likelihood of misclassification of herds and villages. It is unlikely that the FMD vaccines used in Bhutan contained unacceptable levels of NSP residues. Limited field studies in Bhutan by the author (unpublished) showed that FMD-naïve cattle repeatedly vaccinated with double the dose of the same vaccines failed to develop anti-NSP antibodies. However, this has not been validated using a larger sample size for a longer duration and therefore the presence of NSP residues in the vaccines used in Bhutan cannot be completely ruled out.

In conclusion, the study supported the findings of the passive surveillance system that sub-districts/districts bordering India were at higher risk of infection than the interior ones. Serological surveys proved useful in detecting infection that was missed by the passive surveillance system. Cattle are the principal FMD-susceptible species in Bhutan and other species, particularly pigs, appear to play an insignificant role in the epidemiology of FMD in Bhutan. These findings should be considered in further strengthening the FMD control programme in Bhutan.

The study produced baseline epidemiological data on the seroprevalence of FMD in Bhutan. There is a need to undertake further studies, especially on the animal movement patterns (OIE, 2009b), to determine why some districts have a higher seroprevalence than others. The seroprevalence of FMD in the pastoralists or transhumant cattle herds also needs to be studied in order to compare the risks of FMD between the two very different management systems. This is evaluated in the next Chapter where the seroprevalence of FMD in the migratory herds of Bhutan is considered.

The seroprevalence of and risk factors for foot-and-mouth disease in the transhumant herds of Bhutan

7.1 Introduction

Movement of live animals is considered to be one of the single biggest contributors to the spreading of diseases locally, nationally, and globally (Rweyemamu, 1984, Fèvre *et al.*, 2006). Animal movements have often been cited as one of the most common methods of spread of FMD in South-East Asia (Anonymous, 2008b, Wongsathapornchai *et al.*, 2008a) and other parts of the world (Perez *et al.*, 2004, Christley *et al.*, 2005). Knowledge on the species, volume, and routes of animal movements can be useful to predict the patterns of spread of infectious animal diseases (Fèvre *et al.*, 2006). A thorough understanding of the patterns and drivers of animal movement is the key to better management and subsequent reduction in the spread of FMD (Anonymous, 2008b). Animal movements have also been responsible for the introduction of new strains and serotypes of FMDV (Anonymous, 2007e).

Transhumance is the seasonal movement of people with their animals to regions of different climates (Macpherson, 1995). Cattle migration is a deeply-rooted traditional practice of the high-altitude pastoralists/transhumant herders in Bhutan characterised by movement of cattle between cold-temperate regions in the north and warm sub-tropical regions in the south. There is also movement of cattle from the cool-temperate districts to the warm temperate districts in winter. For instance, cattle move from Thimphu (capital

district), which experiences a cool temperate type of climate, to the Punakha district which has a warm temperate climate in winter and vice versa in summer

(<http://www.kuenselonline.com/modules.php?name=News&file=article&sid=13908>,

accessed on 7th November 2009;

<http://www.kuenselonline.com/modules.php?name=News&file=article&sid=15891>

Accessed on 26th June 2010).

The reasons for this migratory practice are multiple such as to avoid the cold harsh winters; for grazing in their sub-tropical grazing lands; traditional practice; and employment opportunities for the herders (Ura, 1993, 2002, Moktan *et al.*, 2008). Most of the pastoralists own pastures or grazing land in the southern part of the country (Ura, 2002). Therefore, every year, tens of hundreds of pastoralists move with their herds in winter to lower altitudes and return back to their villages in the north in early summer. The herders start returning back to their villages by the end of spring when the forage resources in the winter grazing lands become depleted and the weather becomes hot and uncomfortable for the herders and their animals (Ura, 2002). Migratory herds can originate anywhere from the high altitude areas of Haa, Paro, and Bumthang districts and terminate in the sub-tropical areas of Mongar, Lhuentse, Samtse, Sarpang, Zhemgang and Chukha (Ura, 2002). Although, FMD is endemic mostly in the districts located in the sub-tropical zones of Bhutan, in recent years there have been an increasing number of reports of outbreaks in districts located in the temperate zones. For instance, in Bumthang district outbreaks of FMD were recorded in the years 2002 (n=5), 2007 (n=1) and 2008 (n=1) (Anonymous, 2009b). Similarly, Paro and Haa districts, the source of migratory herds, have recorded outbreaks of FMD in the years 2002 and 2003 (Anonymous, 2009b). The districts in the south such as Sarpang, Chukha, and Samtse, that are the destination of most of the

migratory herds, have also had increased numbers of outbreaks over the last few years (Anonymous, 2009b).

The risks posed by the migratory herds in spreading FMD from one zone to another are significant. Many of these herds are known to travel hundreds of kilometres from places located as high as 3500-4000 m.a.s.l. to places as low as 200-300 m.a.s.l. Although FMD has been recorded as an important disease affecting the migratory cattle in Bhutan (Gyaltshen and Bhattarai, 2003, Moktan *et al.*, 2008), no studies have been undertaken to estimate the actual prevalence of FMD and the risk factors associated with disease transmission in the transhumant herds in Bhutan. Questionnaire interviews have been successfully used in pastoral areas elsewhere to understand the livestock husbandry practices and risk factors for FMD in the pastoralist herds (Bronsvort *et al.*, 2003, Bronsvort *et al.*, 2004a, Megersa *et al.*, 2008). Owing to the close association with their livestock, pastoralists are known to possess good knowledge about livestock diseases prevalent in their herds (Bronsvort *et al.*, 2003, Catley, 2006, Bett *et al.*, 2009, Shiferaw *et al.*, 2010).

Therefore, this study was conducted with the following objectives:

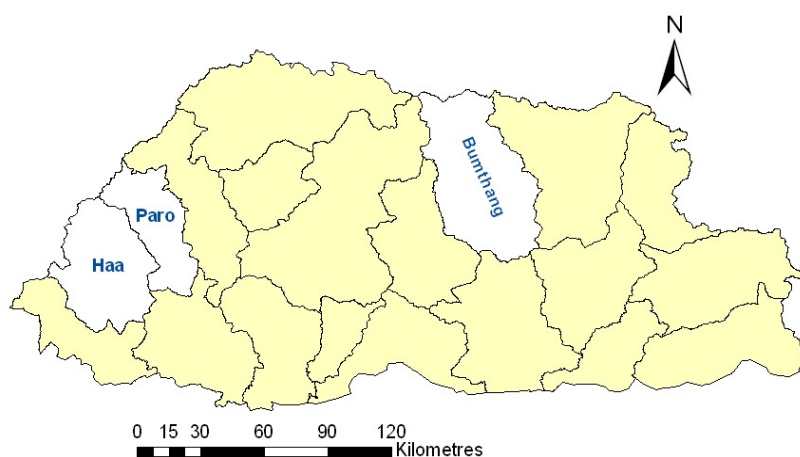
- To understand the livestock management and migratory system existing in the study area.
- To determine the seroprevalence of FMD in the migratory herds of Bhutan.
- To identify and quantify the herd-level risk factors that are associated with the transmission of FMD in migratory herds of Bhutan.

7.2 Materials and methods

7.2.1 Study area

The study was undertaken in the districts of Bumthang (Central Bhutan), Haa and Paro (Western Bhutan). A cross sectional seroprevalence and questionnaire survey was undertaken between August and October 2009 and in June 2010 in these districts (Figure 7.1).

Figure 7.1 Location of the districts for the study reported in this chapter in Bhutan



7.2.2 Sample size

7.2.2.1 Bumthang and Haa districts

With a total of 66 herds migrating and an average of 46 cattle per herd in Bumthang and 72 herds migrating and an average of 30 cattle per herd in Haa (Anonymous, 2009e, 2009d), a 5:1 cost ratio of herds against animals, the programme Survey Tool box version 1.0 beta (Cameron, 1999), gave a two stage sample size of 30 herds and 5 cattle per herd in each of these districts. A total of 150 cattle each had to be sampled from Bumthang and Haa districts to give a 95% confidence level with a precision of $\pm 8\%$ to detect a seroprevalence of 20% at the individual animal level. Since no such studies had been done in the country before, an assumed within-herd variance of 0.20 and a between-herd variance of 0.05 was

used, as has been used elsewhere in South Asia, to determine the necessary sample size (Cameron, 1999).

7.2.2.2 Paro district

Since, no sampling frame was available on the number and location of migratory herds in this district, targeted and convenience sampling was done. Visits were made to the herds during their migration or after their arrival in their originating villages.

7.2.3 Sampling strategy

For Haa and Bumthang, a two-stage probability proportional to size (PPS) sampling was used. In the first stage, herds were selected by the PPS method without replacement. Thus, herds containing more cattle were more likely to be selected than those with fewer cattle. Once herds were selected, individual animals were then selected by simple random sampling using a random table after listing all the animals in the herd. The sample collection, preservation and transportation were done as previously reported (Chapter 6).

7.2.4 Laboratory tests

As the study population had been vaccinated against FMD in the years preceding the survey, a FMD non-structural protein (NSP) 3ABC ELISA, that could differentiate antibodies induced by natural infection from those induced by vaccination, was used (Kitching, 2002a, OIE, 2008). A commercial NSP kit, PrioCHECK® FMDV NS, from Prionics AG (Switzerland), was used as described in Chapter 6.

To assess the immune status of animals vaccinated against FMD, some of the NSP-negative sera originating from cattle from herds with no history of an outbreak of FMD but with a history of vaccination were tested with a commercial structural protein FMD ELISA (PrioCHECK® FMDV Type O) produced by Prionics AG (Switzerland). This is a solid-phase blocking ELISA that detects antibodies to the structural proteins of the FMDV type

O resulting from either vaccination or infection (Chénard *et al.*, 2003). This is a monoclonal antibody-based ELISA containing the strain O1 Manisa of the O serotype and can be used as a screening assay in all domestic species including cattle, sheep and pigs (Chénard *et al.*, 2003). The test has a reported diagnostic specificity of 96% and sensitivity >99% (Chénard *et al.*, 2003). All serological tests were conducted at the National Centre for Animal Health in Bhutan.

7.2.5 Questionnaire survey

A pre-designed questionnaire (Appendix 2; approved by the Murdoch University Human Ethics Committee) was used in this study. The questionnaire was written in English since the enumerators were well versed in English. The questionnaire was pre-trialled on 5 farmers from Bumthang and Haa districts, modified slightly and a final version produced. The questionnaire was applied as described in Chapter 5.

7.2.6 Data analysis

The data management and statistical analysis was done as described in Chapters 5 (for risk factors) and 6 (for seroprevalence). For the univariate analyses, the outcome variable of interest was the “pastoralist-diagnosed FMD in Bhutan”. The multivariable logistic regression was undertaken as described in Chapter 5.

7.3 Results

7.3.1 Study population profile

A total of 80 pastoralists were interviewed from 32 villages located across 8 sub-districts of the three selected districts (Table 7.1). The distribution of the study population (pastoralists) in terms of gender, literacy, and main income source is given in Table 7.2. Overall more males (70%, 56/80) were interviewed than female (30%) and the trend was similar in all districts. The majority (81%, 65/80) of the respondents were illiterate.

Table 7.1 Sampling profile for the risk factor study for the pastoralists

Districts	Sub-districts	Villages	No. of Pastoralists	Percentage of all respondents
Bumthang	3	13	31	38.75%
Haa	3	13	33	41.25%
Paro	2	6	16	20.00%
Total	8	32	80	100.00%

Table 7.2 Profile of the pastoralists (Number and % in parenthesis)

Variables	Bumthang	Haa	Paro	Total
<i>Gender</i>				
Male	21 (67.7)	21 (63.6)	14 (87.5)	56 (70)
Female	10 (32.3)	12 (36.4)	2 (12.5)	24 (30)
<i>Literacy</i>				
Literate	5 (16.1)	7 (21.2)	3 (18.7)	15 (18.8)
Non-literate	26 (83.9)	26 (78.8)	13 (81.3)	65 (81.2)
<i>Main source of income</i>				
Agriculture	8 (25.8)	2 (6.1)	10 (62.5)	20 (25)
Livestock	23 (74.2)	31 (93.9)	6 (37.5)	60 (75)

Livestock farming (75%, 60/80) was considered to be the most important source of income for the pastoralists as compared to agriculture (25%, 20/80). The median land holding per household was 2.02 hectares (range: 0 – 10.1 hectares) and a household was found to consist of, on average, 9 persons (range: 3 – 23). On average, a respondent had lived in the village for 48 years (range: 20 – 72) and had been undertaking livestock farming for an average of 30 years (range: 6 – 71).

7.3.2 Livestock farming system

Cattle (85.8%) were the predominant species reared by the pastoralists followed by horses (7.1%), poultry (4.7%), pigs (1%), yaks (0.9%), goats (0.4%), and sheep (0.1%) (Table 7.3). Only one household had sheep, five households owned goats, and 13 had pigs. Cattle were owned by all of the households. On average, each pastoralist owned 34.5 cattle (± 22.6 SD) and 2.8 horses (± 3.3 SD). Most (96%, 77/80) pastoralists ranked dairy production as the most important reason for rearing cattle.

Table 7.3 Population distribution of livestock species

Species	Bumthang	Haa	Paro	Total	Percent of all animals
Cattle	1283	1005	473	2761	85.8
Yaks	0	30	0	30	0.9
Sheep	4	0	0	4	0.1
Goats	1	1	10	12	0.4
Pigs	0	17	14	31	1.0
Horses	160	54	13	227	7.1
Poultry	39	88	25	152	4.7
Total	1487	1195	535	3217	100.0

Providing draught power (56.3%, 45/80) and manure (36.3%, 29/80) were ranked as the second and third most important purposes for rearing cattle, respectively. However, this was not exclusive and cattle were reared for multiple reasons.

In their village, herders either tethered some cattle near their house (33.8%, 27/80) or allowed them to move freely around their housing compound (82.5%, 66/80) when not sent for grazing. Only one herder kept cattle always housed in a shed during the day. Again, this practice was not stand-alone and within a herd, pastoralists allowed some of their animals to move around while others were tethered in the field. During the night, 56.3% (45/80) of the pastoralists allowed their cattle to graze freely around their house whereas

42.5% (34/80) kept their cattle housed in a shed after the animals returned back from grazing in the forest. Pastoralists who owned sheep, goats, and pigs always kept their animals housed in a shed or pen when not sent for grazing or on migration. The pastoralists lived in villages with clustered houses which provided the opportunity for animals from a village to mix at various places including watering and grazing and also around the housing compound (Figures 7.2 and 7.3).

Figure 7.2 Cluster of houses in Tshebji village, Naja sub-district, Paro district.



Figure 7.3 Cluster of houses in Shari village, Sama sub-district, Haa district



Note the cattle barns/sheds at the back of each house

The animals were managed under a subsistence production system whereby they were allowed to graze freely in the village and forest. The animals were fed with a variety of feedstuffs including commercial feed, crop by-products, kitchen wastes, improved pastures and the leaves from fodder trees. The crop by-products fed consisted of maize grain, paddy straw, rice bran, paddy husk, and maize plant.

The animals' drinking water was obtained from a range of sources including taps (reticulated source), rivers, streams, spring or from irrigation sources. For most farmers tap water constituted an important source of water for both humans and animals (Figure 7.4).

Figure 7.4 Location of tap water at the centre of each cluster of houses



Note: Use of tap water as a source of drinking water for both humans and animals

7.3.3 Prevalence of FMD

The unweighted herd-level prevalence of FMD (Table 7.4), based on the observations of the pastoralists, was 16.3% (95% CI: 8.9, 26.2). However, after weighting (Cochran, 1977, Mercier *et al.*, 2010) for the number of herds available in each district (Table 7.5), the herd-level prevalence of FMD was 20.7% (95% CI: 12.4, 31.2)

Table 7.4 Pastoralist-diagnosed unweighted prevalence of FMD

Districts	No. of villages	No. of villages affected with FMD	Village-level prevalence (%) (95% CI)	No. of herds surveyed	No. of herds affected with FMD	Herd-level prevalence (%) (95% CI)
Bumthang	13	2	15.4 (1.9, 45.4)	31	3	9.7 (2.0, 25.8)
Haa	13	3	23.0 (5.0, 53.8)	33	4	12.1 (3.4, 28.2)
Paro	6	4	66.7 (22.3, 95.7)	16	6	37.5 (13.8, 61.2)
Total	32	9	28.1 (13.7, 46.7)	80	13	16.3 (8.9, 26.2)

Table 7.5 Pastoralist-diagnosed weighted prevalence of FMD

Districts	No. of herds present	No. of herds surveyed	*Proportion of population (a)	No. of herds affected with FMD	Herd-level prevalence (%) (b)	Prevalence after weighting with 95% CI (a x b)
Bumthang	66	31	0.303	3	9.7	2.9 (0.1, 16.2)
Haa	72	33	0.33	4	12.1	4.0 (0.2, 17.2)
Paro	80	16	0.367	6	37.5	13.8 (2.0, 39.9)
Total & weighted average	218	80	1.0	13	16.3	20.7 (12.4, 31.2)

**Weightings using the total herds available in each district as the denominator*

Twenty eight percent (95% CI: 13.7, 46.7) of the villages had reported having at least one outbreak of FMD within the preceding 5-years. There were no significant differences between the three districts for both the village-level prevalence of FMD ($P=0.717$, $\chi^2=0.667$, $df=2$) or the herd-level prevalence ($P=0.395$, $\chi^2=1.857$, $df=2$).

Cattle ($n=249$) were the most common species affected with FMD, being recorded in all affected herds, whereas only two herds reported the occurrence of clinical signs of FMD in pigs. Sheep and goats were not reported to have been affected with FMD in the study area during the 5-year period preceding the survey. Five households reported deaths in cattle (36/249) during the recent outbreak of FMD in their herds. Paro and Haa districts had a higher morbidity rate ($P<0.01$, $\chi^2=131.6$, $df=2$) and case fatality rate ($P<0.01$, $\chi^2=68.7$, $df=2$), than Bumthang (Table 7.6).

Table 7.6 Morbidity and case fatality patterns in cattle due to FMD

Districts	No. of cases in cattle diagnosed by farmers	*No. of cattle	No. of dead cattle	Morbidity rate (95% CI)	Case fatality rate (95% CI)
Bumthang	15	165	0	9.1 (5.2, 14.6) ^a	0.0 (0.0, 21.8) ^c
Haa	55	151	27	36.4 (28.8, 44.6) ^a	49.1 (35.4, 62.9) ^{b,c}
Paro	179	279	9	64.2 (58.2, 69.8) ^a	5.0 (2.3, 9.3) ^b
Total	249	595	36	41.9 (37.8, 45.9)	14.5 (10.3, 19.4)

^{a, b, c} Superscripts with the same letters are significantly different ($P < 0.05$); *Population based on questionnaire survey

7.3.4 Vaccination profile

All pastoralists reported having their cattle vaccinated against FMD during the 5-years preceding the survey. Most (95%, 76/80) reported vaccinating their cattle once a year. Only two farmers (2.5%) vaccinated their animals twice a year and the remaining 2.5% (n=2) vaccinated their cattle once every 2 to 3 years. The benefits given by farmers for adopting vaccination included: not having any outbreaks (78.8%, 63/80) after vaccination; early recovery of affected animals (13.7%, 11/80); and animals not getting the disease even though other animals in the village were affected (7.5%, 6/80).

7.3.5 Livestock movement

When not on migration, the pastoralists grazed their cattle away from their herds and villages. The majority of pastoralists (92.5%, 74/80) sent cattle to the forest for grazing and 25% (20/80) sent cattle to community grazing land. These were not exclusive as animals ended up grazing at various places at different times. The majority (86.3%, 69/80) of the pastoralists allowed their cattle to mix with other herds at these grazing areas.

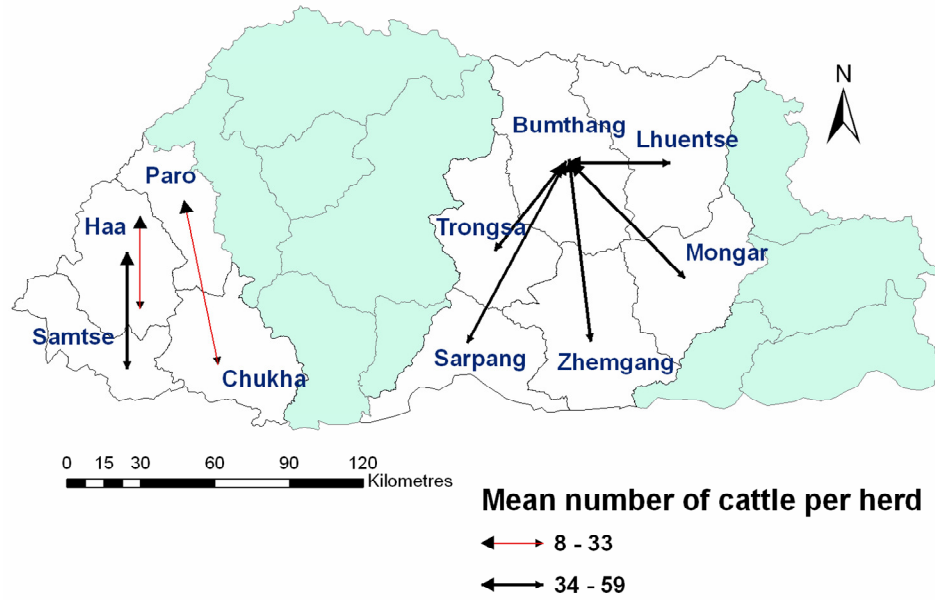
7.3.6 Migration practices in the study area

7.3.6.1 Background

The herders' permanent residences were in villages in Bumthang, Haa or Paro. They owned *tsamdro* (natural pasture) and some agricultural land in the southern villages where they had temporary accommodation. Those owning agricultural land also cultivated paddy fields. They practice a system called *nothue* whereby they had shared ownership of cattle along with other herders in the north (Gyaltshen and Bhattarai, 2003). This is a partnership fostered between the herders for centuries whereby if one party takes the animals on migration to the south, the other party looks after the animals once they are back in their villages in the north. This system of *nothue* is more prevalent in Haa and Paro than in Bumthang.

The pastoralists from Bumthang district migrated to the villages located in the sub-tropical areas of Sarpang, Trongsa, Zhemgang, Lhuentse and Mongar districts. The pastoralists from Haa district migrated to the sub-tropical areas of Samtse district and to other cooler areas within Haa district. The pastoralists from Paro district migrated to the subtropical areas in Chukha district. In Figure 7.5 the overall movement patterns of the migratory herds in the study area are displayed. Pastoralists originated from villages located at a median altitude of 2789 m.a.s.l (range: 2212 - 3300) in the temperate zone and moved to villages located in the sub-tropical zone at a median altitude of 1288 m.a.s.l. (range: 226 - 2206). Pastoralists from Bumthang moved to as low as 1337 m.a.s.l in Nabji village in Trongsa district.

Figure 7.5 Overall migration patterns in the study area at the district level



The lowest altitudes moved to by the pastoralists from Paro and Haa were 226 m.a.s.l in Phuentsholing sub-district and 398 m.a.s.l in Sarkitar village of Samtse sub-district, respectively. The details of the inter-district migration pattern in the study area at the sub-district level are depicted in Figures 7.6 to 7.8.

On average, pastoralists took a median time of six days (range: 1-30) to reach their destination village. The pastoralists from all districts usually commenced their migration in September or October and returned to their villages between April and June (Table 7.7; Figure 7.9). The pastoralists stayed for a median time of 7.5 (range: 3-10) months in their destination villages before returning to their resident villages.

Figure 7.6 Migration patterns within Haa and between Haa and Samtse districts at the sub-district level

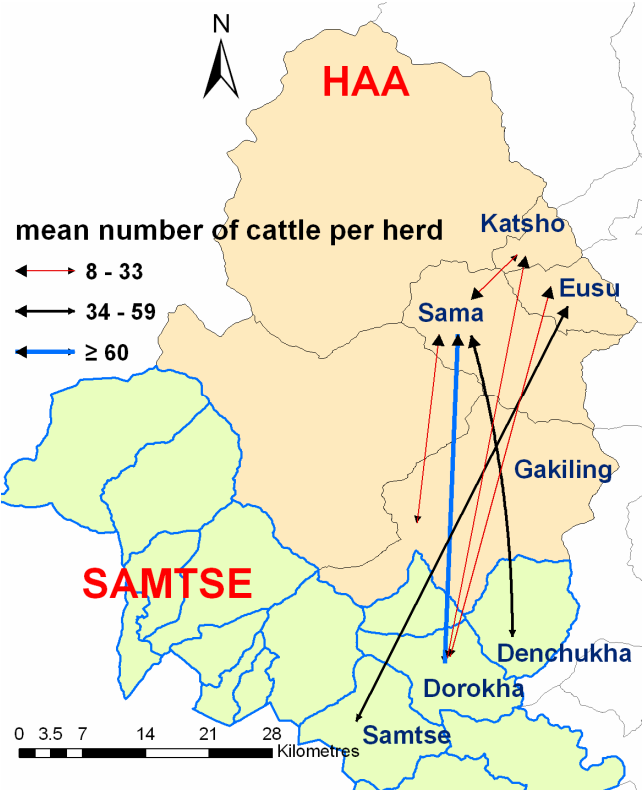


Figure 7.7 Migration patterns between Paro and Chukha districts at the sub-district level

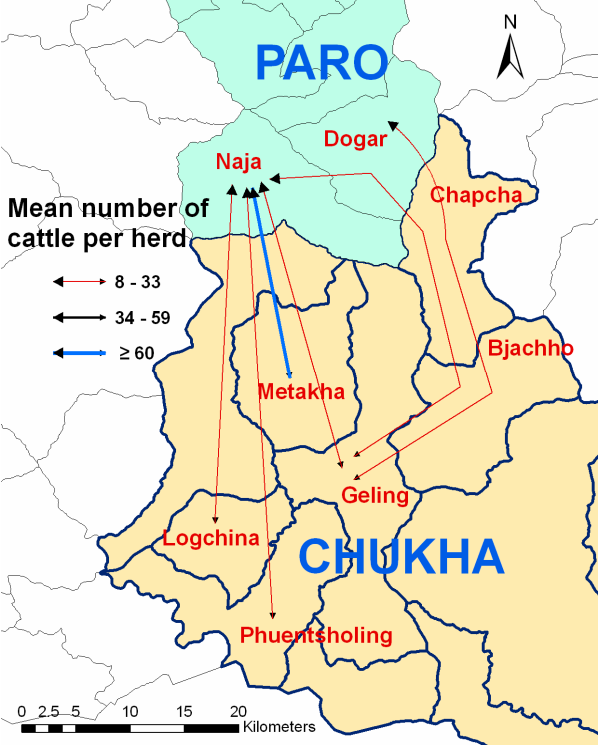


Figure 7.8 Migration patterns between Bumthang and Mongar, Lhuentse, Zhemgang, and Sarpang at the sub-district level

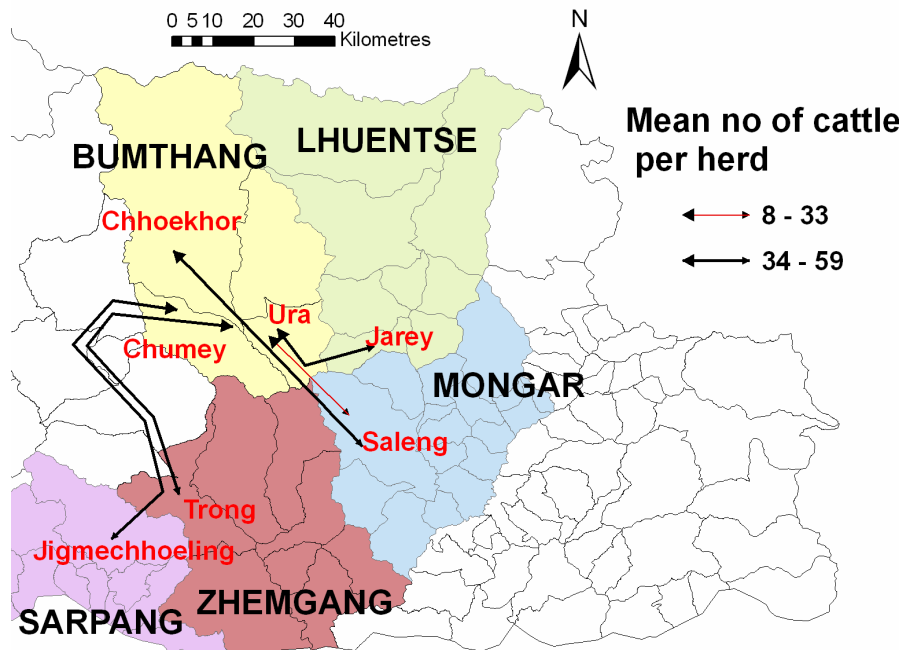


Figure 7.9 A herd of cattle on the move from Trongsa to Bumthang (19 May 2010)



Table 7.7 Duration and months of migration in the study area

Districts	Median no. of days taken for migration (range)	Months migration commenced	Months of return to the resident villages
Bumthang	10.0 (3.0, 30.0)	September, October	April, May
Haa	5.0 (2.0, 20.0)	September, October	May, June
Paro	5.5 (1.0, 7.0)	September, October	April, May
All districts	6.0 (1.0, 30.0)		

Half (50%, 40/80) of the pastoralists ranked shortage of feed and fodder as the main reason for undertaking a migration. Thirty-seven percent (30/80) of the pastoralists ranked avoidance of cold weather as the main reason for migration. Other reasons included better economic opportunities in the sub-tropical areas (3.8%, 3/80) and adherence to traditional practice (8.8%, 7/80). As the means of migration, the majority of pastoralists (96.3%, 77/80) walked their cattle as compared to 3.7% (3/80) who transported their cattle by trucks. The majority of respondents (90%, 72/80) took all of their cattle on migration, irrespective of their breed, gender, and reproductive status. Most herders (81%, 65/80) migrated with other herders from the same district or village. All herders took the same route on their outward and return journeys.

Nearly all pastoralists reported seeing other cattle (91.3%, 73/80) or wild animals (86.3%, 69/80) such as wild pigs and deer during their most recent migration.

7.3.6.2 Diseases prevalent in the migratory herds

Infectious diseases such as FMD, black quarter (black leg), haemorrhagic septicaemia, and foot-rot, were reported by farmers to have occurred in animals during migration. Other diseases noted included plant poisoning, lameness, diarrhoea, dysentery, fever, paralysis, weakness and haematuria. Foot-and-mouth disease was called by different local names in

the three study districts (*Kha-tsi* in Haa, *Ghow* in Paro and *Kha-tsa-Kang-Ney* in Bumthang).

Thirty-five percent (28/80) of the pastoralists reported losing some cattle during their most recent migration. The main cause of deaths during the migration included disease (50%, 14/28) followed by accidents (35.7%, 10/28) and predation by wild animals (5%, 4/80). Most (82.5%, 66/80) pastoralists reported that the number of deaths during migration had decreased over the 10-year period preceding the questionnaire survey. In contrast 7.5% (6/80) of the herders reported an increase in mortality during migration whereas one (1.3%) respondent stated that the mortality trend had remained the same over the last 10 years. Seven respondents didn't know if there had been a change or not.

The decrease in the mortality reported by 65 pastoralists was attributed to the provision of better health care provided by the veterinary services (89.2%, 58/65) followed by a reduction in the number of wildlife predators (10.8%, 7/65).

Ten pastoralists (12.5%) reported seeing outbreaks of FMD in villages while migrating through those villages. Twelve pastoralists (15%) reported outbreaks of FMD in their own herds during migrations undertaken within the 5 years preceding this survey. Of these, eleven continued their journey until they reached their destination while one farmer waited until the affected animals recovered from the disease before continuing.

7.3.6.3 Vaccination before and after migration

The majority (92.5%, 74/80) of the pastoralists reported having their cattle vaccinated against FMD prior to undertaking the migration. On average, animals were vaccinated at least 14 days (range: 1-30) before the commencement of their migration. Only 19 (23.8%) pastoralists revaccinated their cattle before they returned back to their place of residence.

7.3.6.4 Trends in migration

Most respondents (62.5%, 50/80) reported a decrease in the number of herders migrating over the 10 year period preceding this survey. Only one herder (1.3%) reported an increase in the number of herds migrating and 25% (20/80) reported no change in the number of herds migrating during this period. Nine respondents were not sure of the change. All pastoralists reported a shortage of labour as the main reason for a decrease in the migration trend. When asked whether herders would stop the practice of migration in the future, more than half (55%, 44/80) intended to continue this practice whereas 16.3% (13/80) wanted to discontinue. Twenty-nine percent (23/80) of the herders were not sure whether they would stop this practice or not in the future.

7.3.6.5 Knowledge on FMD

The majority of pastoralists (95%, 76/80) could recall some of the clinical signs of FMD. Similarly, the majority (93.8%, 75/80) understood some of the important disease control measures that needed to be applied in the event of an outbreak of FMD in their herd or village. Most (94%, 75/80) herders could correctly name some of the wild animals susceptible to FMD.

7.3.7 Seroprevalence

7.3.7.1 Sampling profile

Blood was collected from 378 cattle belonging to 92 herds of 32 villages in the study area. All these cattle had recently migrated from the sub-tropical villages and were tested on return to their originating villages.

More samples (76%, 287/378) were collected from females than males (24%, 91/378).

More samples were also collected from local breeds (74.1%, 280/378) than

improved/imported breeds (25.9%, 98/378). The median age of the animals sampled was 3 years (range: 0.5 - 13 years).

7.3.7.2 Seroprevalence

The overall test prevalence of FMD at the animal-level and herd-level was 24.8% (95% CI: 20.6, 29.5) and 64.1% (95% CI: 53.5, 73.9) respectively (Table 7.8). The herd-level seroprevalence (Table 7.9), after weighting for the total herds available in each district, was 65.5% (95% CI: 54.9, 75.1)

Table 7.8 Seroprevalence (unweighted) of FMD at the animal- and herd-level

Districts	No. of animals (positive /tested)	Animal-level seroprevalence (95% CI)	No. of herds (positive /tested)	Herd-level seroprevalence (95% CI)
Bumthang	12/160	7.5 (3.9, 12.7) ^a	9/31	29.0 (14.2, 48.0) ^a
Haa	46/140	32.8 (25.2, 41.3) ^b	31/37	83.7 (68.0, 93.8) ^b
Paro	36/78	46.2 (34.8, 57.8) ^b	19/24	79.2 (57.8, 92.9) ^b
Total	94/378	24.8 (20.6, 29.5)	59/92	64.1 (53.5, 73.9)

^{a, b} Superscripts with the different letters are significantly different (P<0.05)

The prevalence of NSP-antibodies at the animal-level varied significantly (P<0.01, $\chi^2=49.5$, df=2) between the districts with Paro recording the highest seroprevalence followed by Haa and Bumthang (Table 7.8).

Table 7.9 Seroprevalence (weighted) of FMD at the herd-level

Districts	No. of herds available	No. of herds surveyed	*Proportion of Population (a)	No. of herds sero-positive	Prevalence (%) at herd-level (b)	Prevalence after weighting with 95% CI (a x b)
Bumthang	66	31	0.30	9	29.0	8.8 (1.7, 24.6)
Haa	72	37	0.33	31	83.7	27.6 (14.2, 44.7)
Paro	80	24	0.36	19	79.2	29.1 (12.6, 51.0)
Total & Weighted average	218	92	1.00	59	64.1	65.5 (54.9, 75.1)

**Weightings using the total herds available in each district as the denominator*

There were significant differences in the unweighted animal-level seroprevalence between Bumthang and Haa ($P < 0.01$, $\chi^2 = 18.7$, $df = 1$); and Bumthang and Paro ($P < 0.01$, $\chi^2 = 11.7$, $df = 1$). The prevalence of NSP-antibodies at the herd-level (unweighted) also varied significantly ($P = 0.002$, $\chi^2 = 12.4$, $df = 2$) between the districts with Haa recording the highest seroprevalence followed by Paro and Bumthang (Table 7.8). There was no significant difference ($P = 0.228$, Mann-Whitney U test, $z = -1.2$) in the median age between the seropositive (3 years; range: 0.5 – 13) and seronegative animals (2.75 years; range: 0.5 – 10). The overall seroprevalence in females (27.5%, 95% CI: 22.4, 33.1) was significantly higher ($P = 0.038$, $\chi^2 = 4.32$, $df = 1$) than in males (16.7%, 95% CI: 9.6, 26). However, there was no significant difference ($P = 0.52$, $\chi^2 = 0.414$, $df = 1$) in the seroprevalence between the local (25.7%, 95% CI: 20.7, 31.3) and improved (22.5%, 95% CI: 14.6, 32) breeds of cattle.

7.3.7.3 Immune status of the vaccinated animals

Of the 175 NSP-negative sera, only 21.1% (37/175) tested positive to the PrioCHECK® FMDV Type O ELISA (Table 7.10). These sera originated from herds vaccinated against FMD approximately one year prior to the collection reported in this Chapter.

Table 7.10 Seropositivity of NSP-negative sera to Structural protein ELISA

Districts	No. of sera positive/tested	Seropositivity (95% CI)
Bumthang	12/89	13.5 (7.2, 22.4)
Haa	23/70	32.9 (22.1, 45.1)
Paro	2/16	12.5 (1.6, 38.3)
Overall	37/175	21.1 (15.3, 27.9)

7.3.8 Logistic regression for risk factor analyses

The results of the univariable analyses between the predictor variables (putative risk factors) and the response variable (“Pastoralist-diagnosed FMD in Bhutan”) are given in

Table 7.11. A total of 34 predictor variables based on biological plausibility were analysed for association with the outcome variable “Pastoralist-diagnosed FMD in Bhutan”. Of these, 18 variables were offered to the multivariable logistic regression ($P < 0.25$) and the best fit logistic regression model is summarised in Table 7.12.

The risk factor study identified three factors that were associated with the occurrence of FMD in the study herds. Pastoralists who sourced water from taps for their cattle were 9.6 times (95% CI: 1.2, 78.3) more likely to report FMD than those whose cattle used other water sources. Pastoralists who allowed their cattle to mix with 6 herds or more from the same village were 6.9 times (95% CI: 1.3, 36.2) more likely to record FMD in their herds than those whose herds mixed with less than 6 herds. The odds of having FMD in a herd increased further (OR=11.3, 95% CI: 1.6, 76.9) when the cattle were allowed to mix with herds from other nearby villages.

Table 7.11 Univariable analysis for “Pastoralist-diagnosed FMD in Bhutan”.

Variable name	Answer	No. +ve/total (% FMD positive)	Odds ratio (95% CI)	P-value
Husbandry related predictor variables				
The main source of income is agriculture?	Yes	3/20 (15)	0.88 (0.22, 3.58)	0.58 ^b
	No	10/60 (16.7)	1.0	
The main source of income is livestock?	Yes	10/60 (16.7)	1.13 (0.28, 4.6)	0.58 ^b
	No	3/20 (15)	1.0	
The main purpose of cattle farming is for dairy production?	Yes	12/77 (15.6)	0.37 (0.03, 4.4)	0.42 ^b
	No	1/3 (33.3)	1.0	
Cattle were tethered in the open air during the day?	Yes	6/271 (22.2)	1.88 (0.56, 6.27)	0.34
	No	7/53 (13.2)	1.0	
Cattle were free ranging during the day?	Yes	10/66 (15.2)	0.66 (0.15, 2.77)	0.41 ^b
	No	3/14 (21.4)	1.0	
Cattle were tethered in the open at night?	Yes	1/4 (25)	1.78 (0.17, 18.56)	0.52 ^b
	No	12/76 (15.8)	1.0	
Cattle were free ranging at night?	Yes	5/45 (11.1)	0.42 (0.12, 1.43)	0.158 ^a
	No	8/35 (22.9)	1.0	
Cattle were housed in a shed at night?	Yes	7/34 (20.6)	1.73 (0.52, 5.7)	0.366
	No	6/46 (13)	1.0	

Table 7.11 (contd) Univariable analysis for “Pastoralist-diagnosed FMD in Bhutan”.

Variable name	Answer	No. +ve/total (% FMD positive)	Odds ratio (95% CI)	P-value
Feeding and watering practices				
Are cattle fed commercial feed?	Yes	6/19 (31.6)	3.5 (1.02, 12.4)	0.038 ^a
	No	7/61 (11.5)	1.0	
Are cattle fed kitchen waste?	Yes	5/16 (31.3)	3.18 (0.87, 11.6)	0.069 ^a
	No	8/64 (12.5)	1.0	
Do cattle graze near the house?	Yes	10/51 (19.6)	2.11 (0.53, 8.4)	0.35 ^b
	No	3/29 (29)	1.0	
Do cattle graze in the forest?	Yes	9/72 (12.5)	0.14 (0.03, 0.67)	0.021 ^{a,b}
	No	4/8 (50)	1.0	
Drinking source sourced from tap.	Yes	6/16 (37.5)	4.88 (1.36, 17.58)	0.01 ^a
	No	7/64 (10.9)	1.0	
Drinking source sourced from river or stream.	Yes	9/32 (28.1)	4.3 (1.19, 15.5)	0.019 ^a
	No	4/48 (8.3)	1.0	
Cattle mix with other cattle herds at water source?	Yes	10/69 (14.5)	0.45 (0.10, 1.99)	0.86 ^b
	No	3/11 (27.3)	1.0	
Mixing of cattle with 1-5 herds at watering points?	Yes	4/48 (8.3)	0.23 (0.06, 0.84)	0.029 ^{a,b}
	No	9/32 (28.1)	1.0	
Mixing of cattle with 6-10 herds at watering points?	Yes	2/7 (28.6)	2.25 (0.39, 13.11)	0.32
	No	11/73 (15.1)	1.0	
Mixing of cattle with >10 herds at the watering points?	Yes	4/14 (28.6)	2.5 (0.65, 9.8)	0.23 ^{a,b}
	No	9/66 (13.6)	1.0	

Table 7.11 (contd) Univariable analysis for “Pastoralist-diagnosed FMD in Bhutan”.

Variable name	Answer	No. +ve/total (% FMD positive)	Odds ratio (95% CI)	P-value
Mixing of cattle with 1 to 5 herds from the same village at grazing	Yes	8/15 (53.3)	13.7 (3.5, 53.6)	<0.0001 ^a
	No	5/65 (7.7)	1.0	
Mixing of cattle with more than 6 herds from the same village at grazing	Yes	8/15 (53.3)	13.7 (3.5, 53.6)	<0.0001 ^a
	No	5/65 (7.7)	1.0	
Mixing of cattle with herds from nearby villages	Yes	10/28 (35.7)	9.0 (2.24, 36.75)	0.001 ^{a b}
	No	3/52 (5.8)	1.0	
Mixing of cattle with other herds from 1 to 5 villages	Yes	4/22 (18.2)	1.2 (0.33, 4.42)	0.74 ^b
	No	9/58 (15.5)	1.0	
Mixing of cattle with other herds from 6 to 10 villages	Yes	5/5 (100)	9.38 (4.87, 18.05)	<0.0001 ^{a b}
	No	8/75 (16.3)	1.0	
Mixing of cattle with other herds from more than 10 villages	Yes	1/1 (100)	6.5 (3.9, 11.08)	0.02 ^{a b}
	No	12/79 (15.2)	1.0	
District influence				
Herds originating from Bumthang	Yes	3/31 (9.7)	0.42 (0.1, 1.65)	0.351 ^b
	No	10/49 (20.4)		
Herds originating from Haa	Yes	4/33 (12.1)	0.58 (0.16, 2.08)	0.542 ^b
	No	9/47 (19.1)		
Herds originating from Paro	Yes	6/16 (37.5)	4.88 (1.36, 17.59)	0.010 ^a
	No	7/64 (16.3)		

Table 7.11 (contd) Univariable analysis for “Pastoralist-diagnosed FMD in Bhutan”.

Variable name	Answer	No. +ve/total (% FMD positive)	Odds ratio (95% CI)	P-value
Herd seropositivity				
Herd seropositive to NSP?	Yes	8/45 (17.8)	1.3(0.38, 4.39)	0.67 ^b
	No	5/35 (14.3)		
Migration factors				
Herd migrates with other herds?	Yes	8/65 (12.3)	0.28 (0.07, 1.03)	0.04 ^a
	No	5/15 (33.3)		
Herd migrates with less than 5 herds	Yes	4/48 (8.3)	0.23 (0.06, 0.84)	0.029 ^{a b}
	No	9/32 (28.1)		
Herd migrates with more than 5 herds	Yes	4/17 (23.5)	1.84 (0.49, 6.94)	0.28 ^b
	No	9/63 (14.3)		
Have seen FMD in villages while on migration?	Yes	5/10 (50)	7.7 (1.83, 32.7)	0.002 ^a
	No	8/70 (11.4)		
Cattle herd size				
Herd size	< 10	0/11 (0)	1.23 (1.1, 1.38)	0.19 ^{a b}
	≥ 10	13/69 (18.8)		

^a variables (with P-values<0.25) were offered to the multivariable logistic regression model. ^b Results of Fisher's Exact test,

Table 7.12 Final logistic regression model

Description of variable	β^a	SE ^b	Wald ^c	Sig ^d	OR ^e (95% CI)
Drinking water sourced from tap	2.26	1.07	4.44	0.035	9.6 (1.2, 78.3)
Cattle mix with more than 6 herds from the same village at grazing	1.94	0.84	5.29	0.021	6.9 (1.3, 36.2)
Cattle mix with herds from other villages at grazing	2.42	0.98	6.14	0.013	11.3 (1.6, 76.9)
Interaction between “Tap water” and “Cattle mixing with more than 6 herds within same village”	5.66	1.96	8.35	0.004	288.5 (6.2, 13469)
Constant	-2.29	1.18	3.77	0.052	

^a β =Estimate of the change in dependent variable attributed to a change of one unit in independent variable

^bSE=Standard error

^cWald=a test that a coefficient is zero based on the Wald statistic

^dSig=Significance for the Wald statistic

^eOR=Odds ratio

The overall model fit was assessed by the Hosmer-Lemeshow test ($\chi^2=4.98$, $P=0.662$, $df=7$) and Omnibus tests of model coefficients ($\chi^2=39.4$, $P=0.000$, $df=5$) as described by Pallant (2005). Between 32.6% and 55.5% variation in the dependent variable could be explained by the set of predictor variables in this model as tested by the Cox & Snell R Square test.

7.4 Discussion

The livestock farming system adopted by the pastoralists was of “free-ranging” type with animals allowed to mix freely at grazing and watering places, both with animals from the

same village as well as animals from other nearby villages. Cattle were the main species of livestock reared. Although, these pastoralists did undertake some agricultural farming, livestock was the principal source of their income. Foot-and-mouth disease was found to be endemic in the study area at varying levels.

The seroprevalence study showed that FMD is prevalent in the migratory herds from as low as 7.5% in Bumthang district to as high as 46.2% in Paro district at the individual animal-level. Similar high seroprevalences of FMD have been reported in other areas of pastoralism (Megersa *et al.*, 2008, Gelaye *et al.*, 2009). The low sensitivity of the “Pastoralist-reported FMD” as compared to the NSP-based seroprevalence could be as a result of sub-clinical infections circulating in the population. As FMD was endemic in these herds (infection-induced immunity) and since vaccination was routinely used, these could have prevented development of frank cases of FMD in the herds.

Herds originating from Paro and Haa had a significantly higher seroprevalence than those from Bumthang. This could be a reflection of the FMD status of the destination sub-districts and villages. Pastoralists from Paro all moved to Chukha to the sub-districts of Phuentsholing, Logchina, Metakha, and Geling. In these sub-districts FMD is endemic with the most recent outbreak recorded in November 2009 (Anonymous, 2009b). Pastoralists from Haa travelled to the sub-districts of Samtse, Dorokha and Denchukha. Foot-and-mouth disease is endemic in Samtse sub-district although no information is available for Dorokha and Denchukha sub-districts (Anonymous, 2009b). In Samtse four separate outbreaks were reported in August 2009. The pastoralists from Bumthang travelled to the sub-districts of Jarey in Lhuentse, Saleng in Mongar, Nabji-Korphu in Trongsa, Jigmechhoeling in Sarpang, and Trong and Nangkhor in Zhemgang. Foot-and-mouth disease is reported sporadically in these sub-districts (Anonymous, 2009b). The last

reported outbreaks of FMD in Nabji-korphu, Jarey, Jigmechhoeling, Trong and Nangkor sub-districts were in the years 2001, 2002, 2002, 2007, and 2007, respectively (Anonymous, 2009b). No outbreaks of FMD were recorded for Saleng sub-district. The lowest altitudes of the destination villages for the pastoralists originating from Paro, Haa, and Bumthang were 226, 398, and 1337 m.a.s.l., respectively. Therefore, herds migrating to relatively lower altitudes and endemic sub-districts had higher seroprevalence than others. This could be due to the higher chance of mixing of migratory animals with the local animals at the grazing ground. Due to the sub-tropical climate, the vegetation is green throughout the year round and grass growth is rapid. Therefore, cattle from the local villages are sent to the forest for grazing throughout the year. Similar findings have been reported in Ethiopia (Megersa *et al.*, 2008) where the seroprevalence of FMD was significantly higher in South Omo zone (370 m.a.s.l.) as compared to other zones located at higher altitudes (2800 m.a.s.l.).

The seroprevalence in the pastoralist herds (24.8%, 95% CI: 20.6, 29.5) was significantly ($P<0.05$) higher than in the sedentary cattle herds (17.5%, 95% CI: 15.6, 19.5; Chapter 6) as has been reported in studies elsewhere (Megersa *et al.*, 2008). In a seroprevalence study of FMD, using the 3ABC NSP test, in the Borana pastoralists in Ethiopia, Rufael *et al.* (2008) also reported seroprevalences of 21% and 55.2% at the animal- and herd-level, respectively, which is similar to the findings of the current study.

Pastoralists who sourced water for their cattle from the tap were at higher risk of reporting FMD in their herds than those that did not. Providing tap water could be a proxy for mixing of herds in the village after they return from grazing, as it is common for animals to congregate in front of the houses. This is supported by the fact that approximately 83% (66/80) of pastoralists allowed their animals to move freely in the village when they were

not sent for grazing. In the villages of Haa and Paro, houses are clustered and usually there is provision of only one watering point (tap) for a group of households. Therefore it is not uncommon for animals to mix frequently at this water source. Studies conducted in other FMD-endemic countries have also identified mixing of animals at water sources as an important risk factor for FMD (Cleland *et al.*, 1996, Bronsvoort *et al.*, 2004a, Oo, 2010).

All pastoralists sent their cattle for grazing every day and this practice allowed the animals to mix at the various grazing areas. Similar observations have been made in the Sagaing Division of Myanmar where cattle sent for grazing to communal grazing lands were at significant risk of FMD infection and this was thought to have been due to close contact between the susceptible animals (Oo, 2010). Similar observations have also been made in Southern Cambodia where cattle sent for grazing in shared grazing areas were at higher risk of infection (Sothyra, 2008).

It is likely that close contact between animals at grazing and watering areas leads to the spread of FMD amongst the transhumant herds, which is similar to the findings reported for the sedentary herds (Chapter 5). In order to prevent the spread of infection from infected to non-infected herds and villages, it is important to create awareness about the need to adopt basic biosecurity measures by the pastoralists. For instance, quarantining of early cases in the index herd or village can substantially reduce the risk of disease transmission both within and between the villages (Cleland *et al.*, 1996).

Although not significant in the logistic regression analysis, in the univariable analysis it was found that some migratory practices were associated with infection. For instance, pastoralists migrating together with more than five herds were at higher risk (OR=3.1, 95% CI: 0.93, 10.7) of infection than those migrating with five or less herds. Similarly, those pastoralists who reported seeing outbreaks of FMD in the villages while on migration were

1.9 times more likely to report FMD in their herds. These practices are likely to contribute to the risk of infection while on migration and subsequently increase the risk to animals in the villages after the farmers return from their migration.

The random effect of district as a potential confounder was checked by forcing this variable into the model. However, no significant effect was seen in the final model. There was significant interaction between the variables “Tap water” and “mixing with 6 or more herds”. This could be due to the fact that animals sourcing water from taps could also be mixing with other animals within the same village as explained above.

Although the majority of the pastoralists reported vaccinating their cattle against FMD, the findings from the study on the animal’s immune status did not indicate adequate protection. The fact that only 21% of the vaccinated animals tested positive to the structural protein test indicates low vaccination coverage. This means that a large proportion of animals would be susceptible to infection if they come into contact with the virus. This could be as a result of either ineffective vaccination or long intervals between vaccinations. Most of these herds were vaccinated more than one year prior to sampling as per the records maintained by the local veterinary offices.

Since this study was confined to three districts, extrapolation of results from this study to the entire country has to be done with caution. However, the livestock production and management system in other pastoral areas in Bhutan is expected to be similar and therefore findings from this study should be representative of the situation in other transhumant areas of Bhutan. Given the difficulty in tracking down the herders because of their highly mobile nature, it was not possible to collect more samples in this study. As with all questionnaire-based studies, some of the disadvantages include the influence of recall bias, interviewer bias and failure to validate the questionnaire responses by repeating

the questionnaire survey among the same respondents (Bronsvoort *et al.*, 2003). Recall bias is less likely to occur as FMD produces characteristic clinical signs, especially in cattle, although the disease could be easily missed in small ruminants (Davies, 2002). Cattle constituted the majority of the FMD-susceptible species in the study area and therefore the disease could be easily detected by the pastoralists. This was supported by the questionnaire data that most of the pastoralists (92.5%, 74/80) could recognise pictures of FMD or could remember (95%, 76/80) some of the key clinical signs of FMD. In a study on the epidemiology of FMD in the Adamawa province of Cameroon, Bronsvoort *et al.* (2003) reported that 69.3% of the herdsmen correctly identified photographs of FMD lesions in the photographs shown to them. Rufael *et al.* (2008), in a study of FMD in Borana pastoral system in Ethiopia, also reported high positive predictive values (93.1%, 95% CI: 78, 98.1) for the pastoralist's diagnosis of FMD on a herd basis when compared with a "gold standard" of the 3ABC ELISA. The authors also reported that the pastoralists had good knowledge about the clinical and epidemiological features of FMD, consistent with modern veterinary thinking (Rufael *et al.*, 2008).

Recommendations

Given the fact that the tradition of seasonal long-distance livestock migration is likely to remain for many years to come, there is a need to develop disease control strategies suited to the needs of this community. The following measures are needed to reduce the risk of infection in the transhumant herds and other herds in the villages where these herds originate from.

Programme to improve awareness on basic biosecurity measures

Considering the fact that the risk factors were all husbandry-related, pastoralists need to be made aware of the basic biosecurity measures to be adopted to reduce the risk of disease transmission within and between the villages. Pastoralists should be made aware of practices such as quarantining of early cases, avoiding mixed grazing, especially during disease outbreaks, and disinfection of the infected premises to reduce the risk of disease transmission.

Enhanced surveillance (clinical and serological)

Serological and clinical surveillance needs to be undertaken in the transhumant herds several weeks before they migrate to identify any sub-clinical infection so that the risk for disease transmission can be reduced. Upon return the herds should be subjected to sero- and clinical-surveillance to again identify any sub-clinical infection. This would ensure that any sub-clinical infection is detected to prevent the further spread to other in-contact herds either while on migration or in the village of origin.

Vaccination

There is a need to undertake effective vaccination of all animals at least 21 days before they begin their migration. The vaccination coverage achieved at the moment is quite low and may not protect the animals from FMDV infection. A separate study is needed to evaluate the effectiveness of vaccination/vaccine in the pastoralist herds in order to provide correct information on the time and frequency of vaccination.

Incentives to reduce migration

Some of the herders were interested in discontinuing migration if incentives were provided by the government. A shortage of fodder is one of the main reasons for migration and therefore leasing land for fodder development could be one option to discourage

pastoralists from undertaking migrations. Another strategy could be reducing the cattle herd size by exchanging unproductive or local breeds of cattle with improved exotic dairy breeds such as Jersey cattle. With reduced herd size, the pastoralists would be able to better manage their animals and reduce the practice of mixed grazing or watering, and still increase production. In any case, a separate study is warranted to look at these options to reduce the practice of migration and to develop better herd health management.

More research

This study was limited by time and resources and therefore the study could cover only a limited area and sample number. In order to understand the epidemiology of FMD in the transhumant herds of Bhutan, it is essential that similar risk factor studies be undertaken in other pastoral areas in the country. It is important to document the animal movement patterns in the transhumant areas to predict and prevent future outbreaks of diseases.

In conclusion, FMD was prevalent in the pastoralist herds at varying intensities. The seroprevalence in the migratory herds was significantly higher than in the sedentary herds thus highlighting the potential risk posed by these herds in the transmission and persistence of FMDV in Bhutan. The findings from the risk factor study complemented those from the sedentary herds and highlighted the risks posed by this production system in the spread of FMD in Bhutan.

In light of the findings reported in this Chapter, there is a need to reorientate the FMD prevention and control programme to cater to the specific needs of the pastoralists of Bhutan. There is also a need to undertake longitudinal studies for at least 3-4 years in order to understand the dynamics of movement patterns that could change over the period of time.

Foot-and-mouth disease is most commonly spread by direct contact between an infected and a susceptible animal (Kitching *et al.*, 2005) and the movement of live animals is considered to be one of the single biggest contributors to the local, national, and global spread of the disease (Rweyemamu, 1984, Fèvre *et al.*, 2006). In the next Chapter the animal movement patterns in an FMD-endemic and FMD-free district are discussed to further the understanding of the epidemiology of FMD in Bhutan.

A study on the livestock trading and animal movement patterns in Sarpang and Tsirang districts to understand the epidemiology of FMD in Bhutan

8.1 Introduction

Foot-and-mouth disease is most commonly spread by direct contact between an infected and a susceptible animal (Kitching *et al.*, 2005) and the importance of the movement of live animals has been highlighted previously (Rweyemamu, 1984, Fèvre *et al.*, 2006). Knowledge on the species, volume, and routes of animal movements can be used to predict the pattern of spread of infectious animal diseases (Fèvre *et al.*, 2006). An understanding of the patterns and drivers of animal movement is the key to better management and subsequent reduction of the spread of FMD (Anonymous, 2008b).

In Bhutan, animal movements occur in various forms. In the villages, owing to the extensive system of livestock management, animals are grazed in the forests, vacant land or community pasture land and are watered communally at nearby streams, rivers or springs (Chapters 5 and 7). The practice of buying and selling of animals, mainly for draught and breeding purposes, also results in the movement of animals, within and between villages and districts. Farmers hire or rent out draught animals for preparation of fields during the agricultural season (Chapter 5). There are also mini-migration systems of animal movements where animals are moved shorter distances from their villages in search of feed and fodder (Ura, 2002). These are the “non-traditional” movements and are usually restricted to within the same district and within the same agro-ecological zone (Ura, 2002).

The “traditional” system of animal movement is described in terms of the animal movements undertaken by the pastoralists living in the high altitudes (more than 2500 m.a.s.l.) wherein animals are moved from one agro-ecological zone (temperate zone) to another (usually sub-tropical zone) for various reasons including: the avoidance of the cold harsh winter; to use the richer grazing pastures in the south; and for socio-economic reasons (Ura, 2002, Gyaltshen and Bhattarai, 2003, Moktan *et al.*, 2008). The dynamics and epidemiological importance of the migration system in Bhutan has been covered in Chapter 7.

Movement of animals within and between villages and districts poses significant risks for the transmission of diseases, such as FMD (Cleland *et al.*, 1995b, Perry *et al.*, 2002). Therefore, in order to understand the epidemiology of FMD in Bhutan, it is essential to understand the livestock trading practices and patterns of animal movements. Currently, this information is not available. Therefore, a comparative study on the drivers and patterns of animal movements in an FMD-endemic and in a FMD-free district is presented. Questionnaires (Cleland *et al.*, 1996, Bates *et al.*, 2001, Marshall *et al.*, 2009) and focus group discussions (Perry *et al.*, 2002, Bett *et al.*, 2009) have been used elsewhere to understand animal movement patterns in other countries. Collective opinions of the village headman and senior farmers in the villages have also been used to understand the animal movement patterns in other FMD-endemic countries (Cleland *et al.*, 1996).

Tsirang district, although surrounded by three FMD-endemic districts, has not had a reported outbreak of FMD during the last 11 years. In contrast FMD is endemic in Sarpang district (Anonymous, 2009b). The dynamics of animal movements in these two districts was compared to identify differences that could explain the variation in disease status between the two districts.

Therefore, the study described in this chapter was conducted with the following objectives:

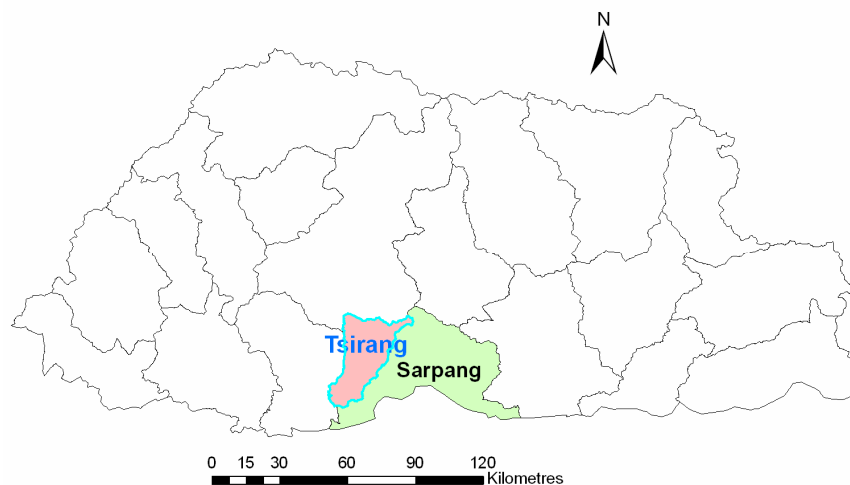
- To document the livestock trading practices and animal movement patterns in these two districts.
- To differentiate the animal movement patterns between an endemic district and a disease-free district so as to understand the reasons for the variation in disease occurrence in these districts and sub-districts within each district.

8.2 Materials and methods

8.2.1 Study Area

The study was conducted in Sarpang and Tsirang districts of Bhutan (Figure 8.1).

Figure 8.1 Location of the study area



8.2.2 Data collection and analyses

8.2.2.1 Data from passive surveillance system

Information on the livestock population in each district was obtained from DOL (Anonymous, 2009a). The list of slaughter houses, quarantine stations, and livestock traders was obtained from the local veterinary office. The number and species of animals moving between the districts was obtained from records maintained by BAFRA.

8.2.2.2 Active data collection

Questionnaires (Appendices 3 and 4) were used to interview the livestock traders, *Gup* (head of a sub-district), *Mangmi* (deputy head of a sub-district), *Tshogpa* (village

headman) and other senior farmers of the sub-districts. The information presented in this Chapter is based on the opinions of 36 livestock traders; 24 heads of the sub-districts; 24 heads of the villages; 24 livestock extension agents; senior farmers, and the district livestock officials. The opinions of the *Gup*, *Mangmi*, *Tshogpa* and the sub-district livestock officials were used to draw the route and volume of animal movements between the sub-districts for the purpose of breeding and rearing. The opinions of the traders were used to map the route and volume of livestock movements for the purpose of slaughter. A two-day focused group discussion was held in each district in which the respondents were briefed about the aims and objectives of the study and the blinding techniques used to protect their identities. Data on the species, volume and direction of movement of animals between sub-districts and between districts were collected through structured questionnaires as well as through focused group discussions. Information was also collected through informal discussions with the livestock traders, senior farmers and government officials working in the sub-districts.

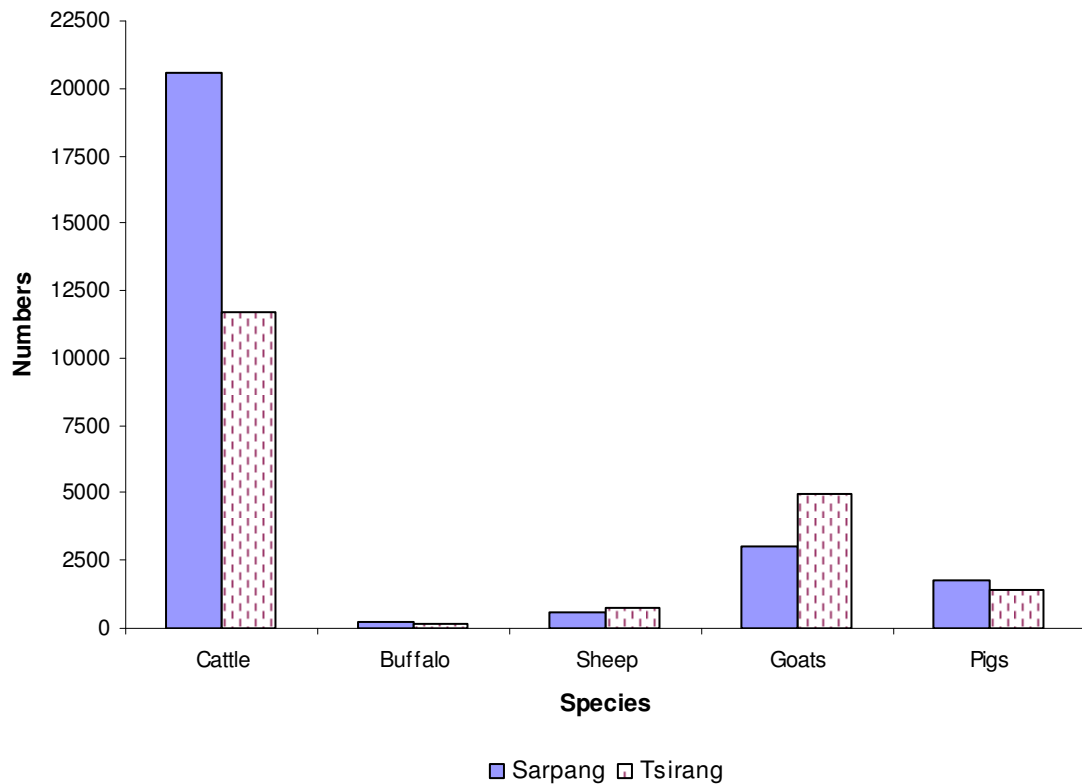
For the purpose of this Chapter the daily movement of animals within sub-districts and villages for grazing and watering purposes was not considered based on the premise that livestock management is done in an extensive system in the study area (Chapter 5) and it was not possible to record all movements. The focus of this Chapter is on livestock movements between sub-districts and districts, as the greatest risk of disease introduction and spread comes from animals moving long distances.

8.3 Results

8.3.1 Livestock farming system in the study area

Cattle (Anonymous, 2009a) are the main species reared, although other species, such as buffalo, goats, pigs, and sheep, are also reared (Figure 8.2).

Figure 8.2 Distribution of FMD-susceptible species in the study area in the year 2007



Source: (Anonymous, 2009a)

The farmers reared male cattle mainly for draught purposes and females for milk and breeding. Goats were reared mainly as a source of cash income as well as for meat during festivals. Goat meat is a delicacy especially for the communities originating from the southern part of Bhutan. Sheep rearing is on the decline (Anonymous, 2004, 2007b) and they are reared mainly for wool. Pigs are reared in small numbers (range: 1 to 10) mainly for fattening purposes and as a source of cash income.

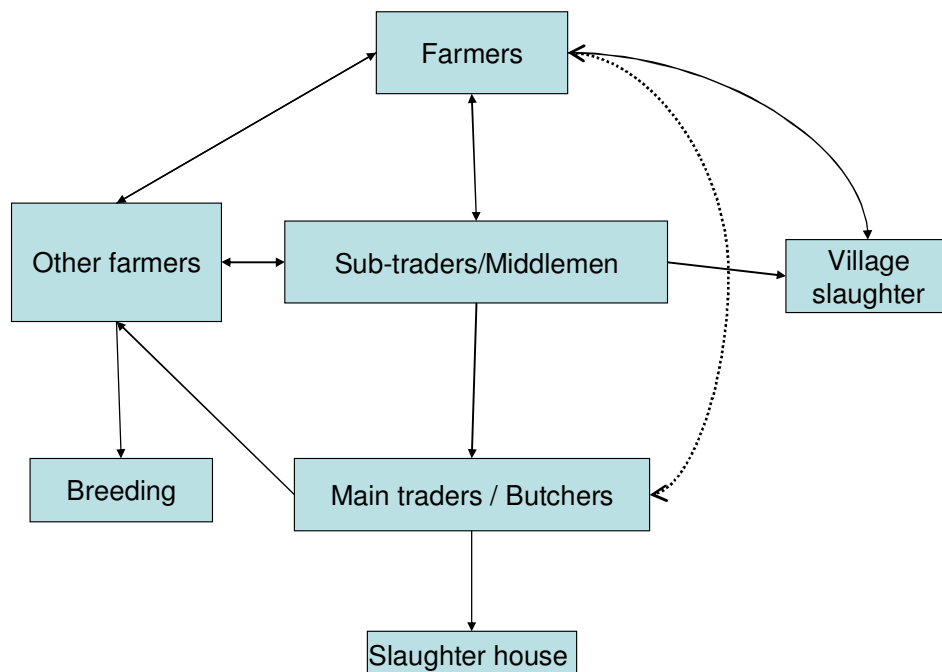
8.3.2 Livestock trading system in the study area

The livestock trading system in the study area is not well structured. There are no organised livestock markets for the sale of live animals (District Livestock Officers, Tsirang and Sarpang, personal communications). Farmers in general preferred to sell their livestock to those people who they believed would not slaughter their animals or sell them to butchers (DLO, Sarpang, personal communication). This was mainly due to religious

sentiments and their close bonding with their animals. The farmers sold their unproductive cattle for slaughter. Sometimes, they sold their entire herd including calves, heifers, and cows.

The stakeholders in the livestock trading system of Bhutan consist of the farmers, sub-traders or middlemen, and the main traders or the butchers as detailed in Figure 8.3.

Figure 8.3 A flowchart showing the livestock trading system in the study area

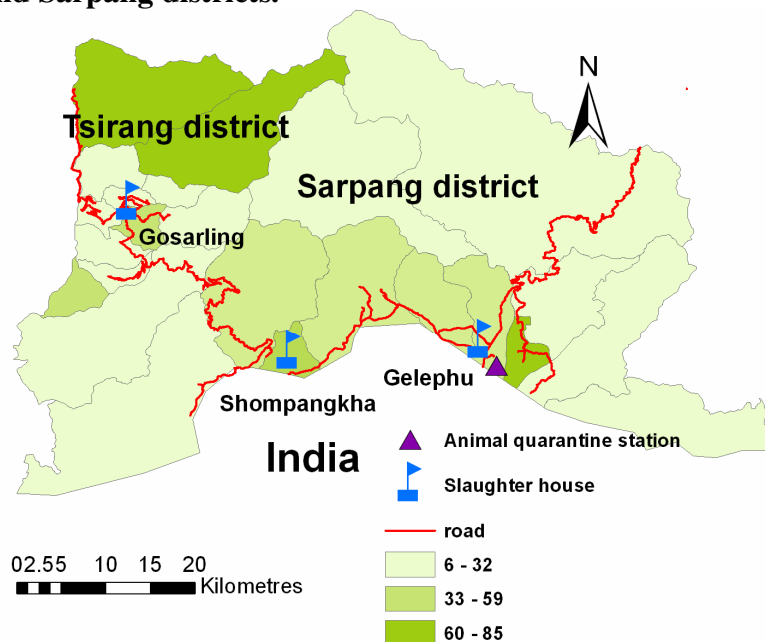


The sub-traders or middlemen are important links in the livestock trading system as they connect the sellers (farmers) with the buyers (butchers) or other farmers. The majority of the sub-traders (28/30) worked part-time as compared to the town traders (6/6) who all worked as full-time traders. All sub-traders (30/30) were farmers and most owned livestock (28/30).

None of the middlemen had licenses to operate their business whereas the butchers were all licensed traders. The butchers were mainly involved in the slaughter of cattle in officially designated slaughter houses (Figure 8.4) whereas the sub-traders often slaughtered small

ruminants and pigs in the villages without proper hygiene/facilities. This was done with or without the approval of the regulatory authorities.

Figure 8.4 Location of slaughter houses and animal quarantine stations in Tsirang and Sarpang districts.



Note: the intensity of green colouration indicates the cattle population for each sub-district per square km

The majority of traders traded one species only due to religious or social restrictions. For instance, the butchers working at the slaughter houses in Gelephu and Shompangkha in Sarpang district, and Gosarling sub-district in Tsirang only dealt with cattle. Sixteen traders traded only cattle; five only goats; six only pigs; six cattle and goats; one cattle and pigs; and two traders traded all three species. The main traders paid some commission or fee to the sub-traders to source suitable animals. The sub-traders then visited the households in the villages to purchase suitable animals.

Cattle were mostly traded in the drier season from October until May (14 traders). The reasons given for this included: easy transportation (no road blocks and easy to cross rivers); end of agricultural season and therefore availability of time for the sub-traders; and more demand for meat during this period due to festivals. Cattle were traded (n=13) less

frequently in summer (June until September) because of frequent road blocks and this time coincided with the busy agricultural season.

The general trend of livestock trading in both districts was that farmers sold their old and unproductive cattle, mostly bulls, at the end of the ploughing season i.e. October until December. The next wave of buying and selling of cattle started from February until April when farmers required young bulls to plough their fields in preparation for paddy cultivation. The farmers bought bull calves and reared them for draught purposes. Once the bulls were old or if the farmers were in need of urgent cash, they then sold these animals and used the money to buy some more calves to rear and to replace the sold animals.

For goats, the trading season was in tandem with the start of the festivals in southern Bhutan between October and December when there is a high demand for goat meat. The trading of goats outside the study area was spurred by demands for goat meat by the *Lhotshampa* community (southern Bhutanese) living in other districts such as Wangdue Phodrang, Thimphu and Punakha. For pigs, the trading season was in tandem with the onset of the Bhutanese traditional New Year starting sometime in February and just before the meat ban which is imposed in the middle of February. A lot of the trading of animals occurred between farmers, without the involvement of middlemen, for rearing purposes. There was virtually no export of live animals to other countries.

8.3.3 Livestock trading practices in Sarpang district

The profile of the Sarpang District, in terms of geo-physical and agricultural practices, has already been discussed in Chapter 4. The district has two slaughter houses, one each in the sub-districts of Gelephu and Shompangkha, and an animal quarantine station in Gelephu (Figure 8.4). The traders (n=19) were in the business for a median of 3 years (range: 1-10).

The traders either purchased animals to be slaughtered or sold them to other traders or farmers. Each sub-district had at least one sub-trader.

On average, a trader traded a minimum of 50 and a maximum of 100 cattle per year (Table 8.1). On average, a full-time trader traded more cattle than a part-time trader (Table 8.1).

The volume of goats and pigs traded are detailed in Table 8.1.

Table 8.1 Number (Median) of animals traded annually per trader by species in Sarpang

Species	Category of traders	Minimum (range)	Most likely (range)	Maximum (range)
Cattle	Full-time	300 (90-1000)	375 (150-1100)	550 (200-1200)
	Part-time	10 (2-100)	25 (3-150)	40 (5-250)
	Both	50 (2-1000)	70 (3-1100)	100 (5-1200)
Goats	Full-time	83 (15-150)	95 (30-160)	113 (45-180)
	Part-time	5 (5-10)	13 (10-20)	20 (15-30)
	Both	10 (5-150)	20 (10-160)	30 (15-180)
Pigs	Both	200 (4-250)	300 (5-300)	400 (7-400)

The majority (14/19) of traders walked their animals when taken for sale to other farmers or traders, even when a road was available. Less than half (9/19) of the traders trucked animals while some did both. The majority (11/19) of the traders always kept their purchased animals in their villages before they were sold. Cattle were slaughtered in the slaughter houses in Shompangkha and Gelephu, whereas goats and pigs were taken to the border villages with India for slaughter and then the meat was brought back for sale in Bhutan. Goats and pigs were also slaughtered in the villages with or without the approval of the regulatory authorities.

The price of animals traded depended mainly on their physical condition and health status.

The average price for each species traded is summarised in Table 8.2.

Table 8.2 Median price (in Ngultrums) per adult animal for each species in Sarpang district

Species	Minimum (range)	Most likely (range)	Maximum (range)
Cattle	5500 (5000-12000)	6500 (5500-15000)	10000 (6000-17000)
Goats	1500 (1500-2000)	2000 (1700-2500)	3000 (2000-3000)
Pigs	7000 (4000-7000)	7500 (7000-8000)	9000 (8500-11000)

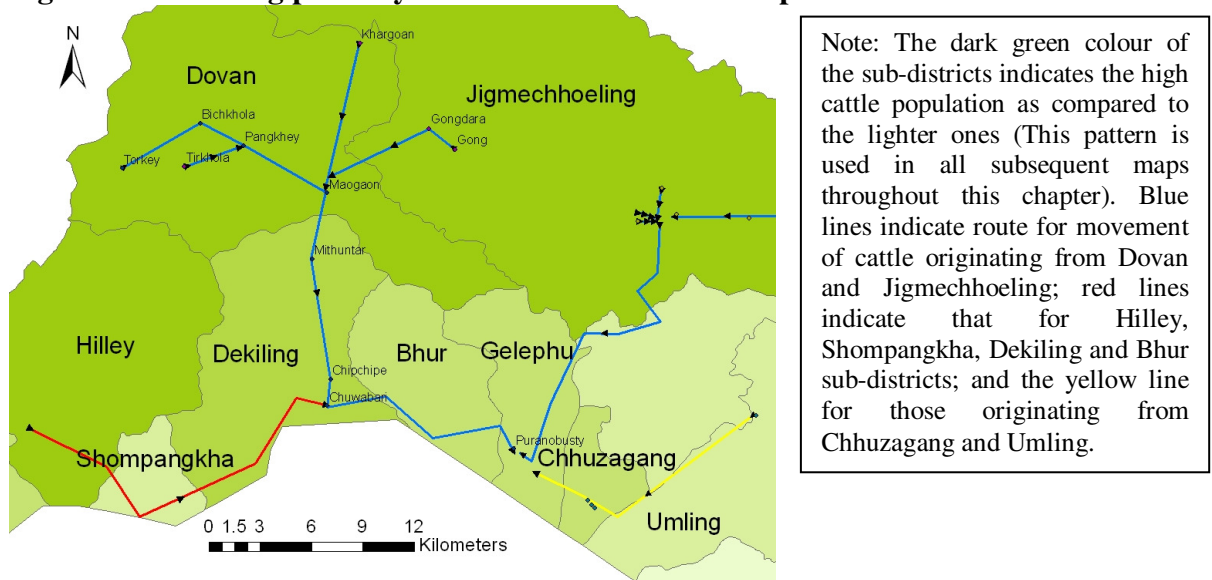
Note: 1US \$ = 45 Nu as at the time of the study (February-March 2010)

Most traders (13/19) reported that farmers wouldn't sell a sick animal. The majority (18/19) of the traders could recognise FMD when shown pictures of the disease and 17 traders reported having heard about the disease. The traders were aware of the need to get official approvals for movements of livestock and livestock products from the local livestock authorities.

8.3.3.1 Cattle movement pathways in Sarpang

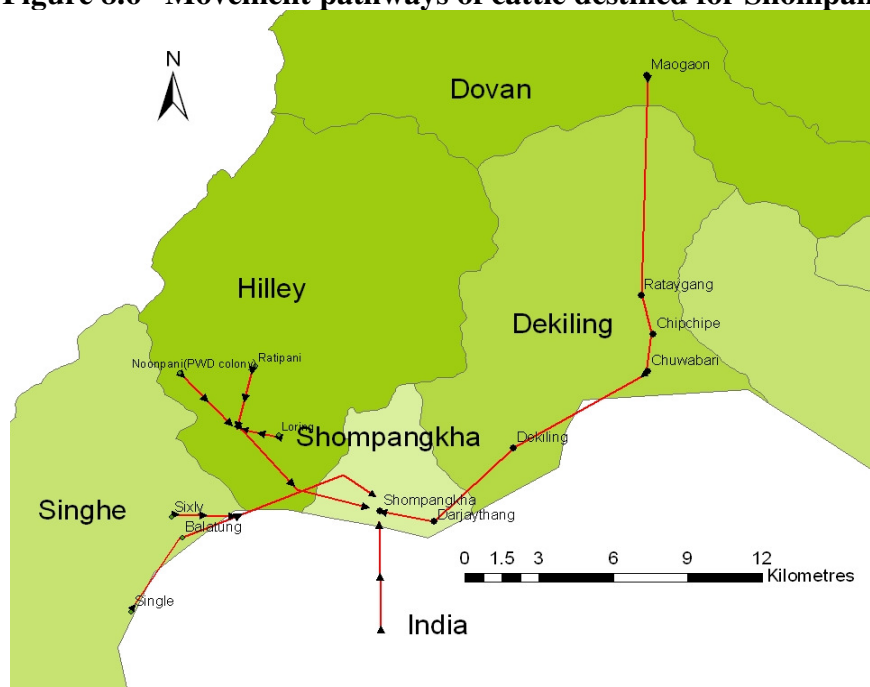
The slaughter houses in Gelephu and Shompangkha were the two main destination points for cattle sold by farmers for slaughter. The majority of cattle originated from Jigmechhoeling and Dovan sub-districts, owing to the high cattle population and low religious sentiments in these sub-districts (Figure 8.5).

Figure 8.5 Trading pathways of cattle destined for Gelephu sub-district



For Dovan sub-district, animals originating from various villages ultimately moved through Maogaon village to reach Gelephu. Cattle were walked until Chuwabari village, Dekiling sub-district, from where the sub-traders handed over the consignment to the butchers who then either walked or trucked them along the main highway to Gelephu. In Jigmechhoeling sub-district, cattle were gathered by the middlemen up to the road point from where they were either walked or trucked along the Gelephu-Trongsa highway to Gelephu. Cattle from Chhuzagang and Umling sub-districts were walked up to Maokhola (a river separating Gelephu from other sub-districts) from where the butchers took over. Traders based in Dovan and Jigmechhoeling also purchased cattle from the neighbouring districts of Tsirang, Wangdue Phodrang, Dagana and Zhemgang for slaughter in Gelephu. The movement pathways for cattle destined for Shompangkha sub-district are shown in Figure 8.6.

Figure 8.6 Movement pathways of cattle destined for Shompangkha sub-district.



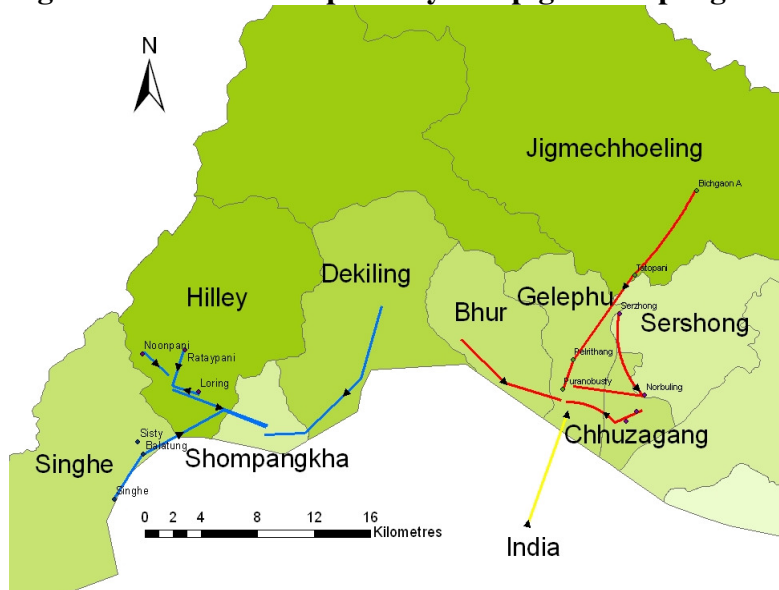
8.3.3.2 Pig trading pathways in Sarpang

The pathways used in the trading of pigs also followed a similar pattern to cattle with animals moving towards either Gelephu or Shompangkha sub-districts (Figure 8.7). A significant finding was that there were movements of live pigs into the Gelephu sub-district from India for slaughtering, as well as for breeding purposes.

8.3.3.3 Goat trading pathways in Sarpang

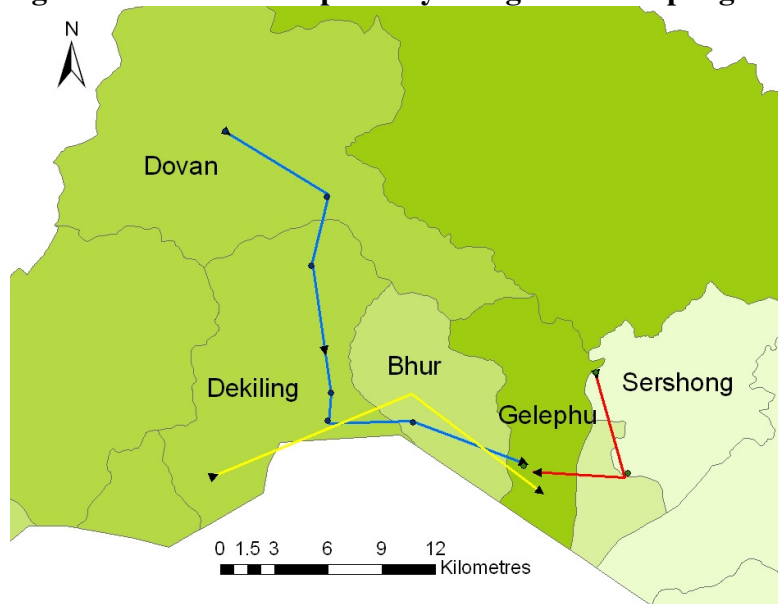
Trading of goats was found to be minimal as compared to cattle and pigs and movements originated from only a few sub-districts (Figure 8.8).

Figure 8.7 Movement pathways for pigs in Sarpang.



Pigs originated from neighbouring sub-districts of Jigmechhoeling, Sershong, Chhuzagang, and Bhur (red line). Another route originated from Singhe, Hilley and Dekiling sub-districts and terminated in Shompangkha for slaughter (blue line). There were also inward movements of pigs into Gelephu sub-district from India (yellow line) through both legal and illegal routes.

Figure 8.8 Movement pathways for goats in Sarpang.



8.3.4 Livestock movement patterns between the sub-districts

8.3.4.1 Livestock movement patterns in Gelephu sub-district

There were significantly more inward than outward movements for this sub-district especially originating from Dovan (Figure 8.9). Similarly for goats, there were also more inward than outward movements (Figure 8.10). In contrast, there were more outward than inward movements for pigs (Figure 8.11).

Figure 8.9 Cattle movements between Gelephu and other neighbouring sub-districts.

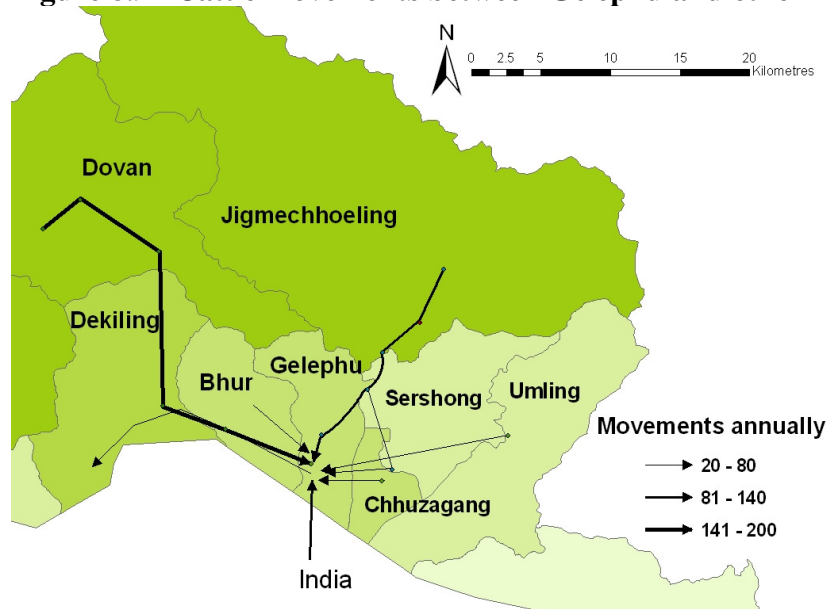


Figure 8.10 Movement pathways for goats between Gelephu and other sub-districts in Sarpang

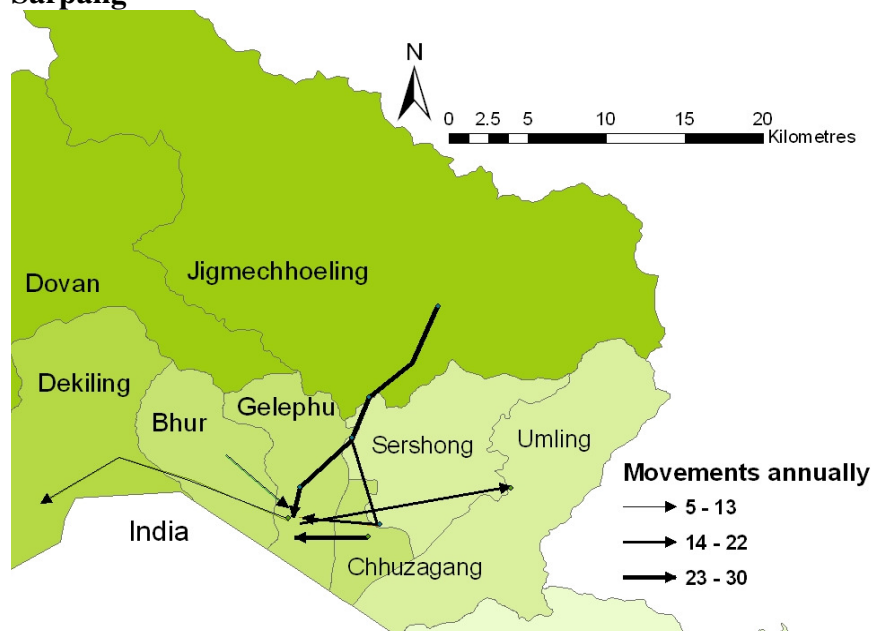
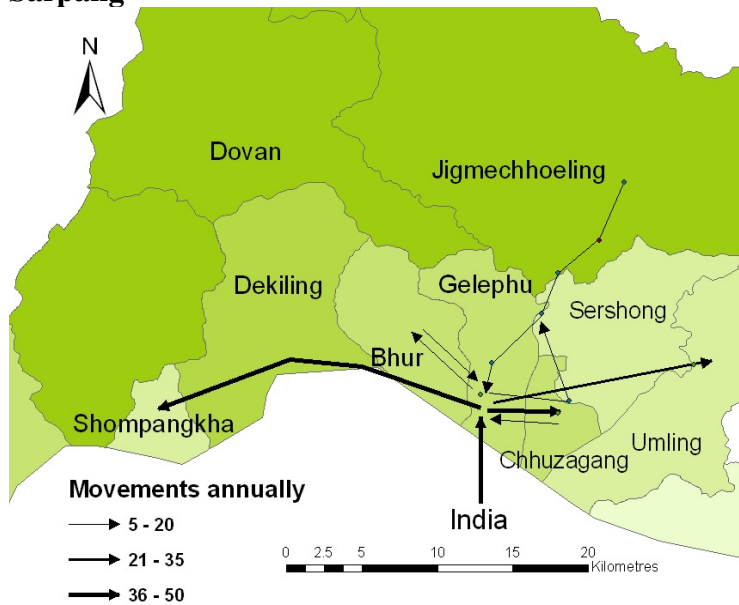


Figure 8.11 Movement pathways for pigs between Gelephu and other sub-districts in Sarpang



Some of the farmers borrowed or lent bullocks to the farmers in India for field preparation, particularly during the paddy plantation season in June-July.

8.3.4.2 Livestock movement patterns in Shompangkha sub-district.

There were more inward than outward movements of cattle for this sub-district (Figure 8.12). Compared with cattle, there were limited movement of goats and pigs in this sub-district (Figure 8.13).

8.3.4.3 Livestock movement patterns in other sub-districts.

For Jigmechhoeling sub-district the movement of cattle was all outwards towards the sub-districts of Gelephu, Sershong, Umling, Dovan, Chhuzagang and Bhur through several routes. The farmers sold crossbred Mithun (*Bos frontalis*) to farmers in Trongsa, Zhemgang and Bumthang. Farmers also exchanged these breeds of cattle for Brown Swiss cattle or horses from the farmers of the above three districts (*Gup*, Jigmechhoeling sub-district, personal communication).

Figure 8.12 Cattle movement patterns in Shompangkha

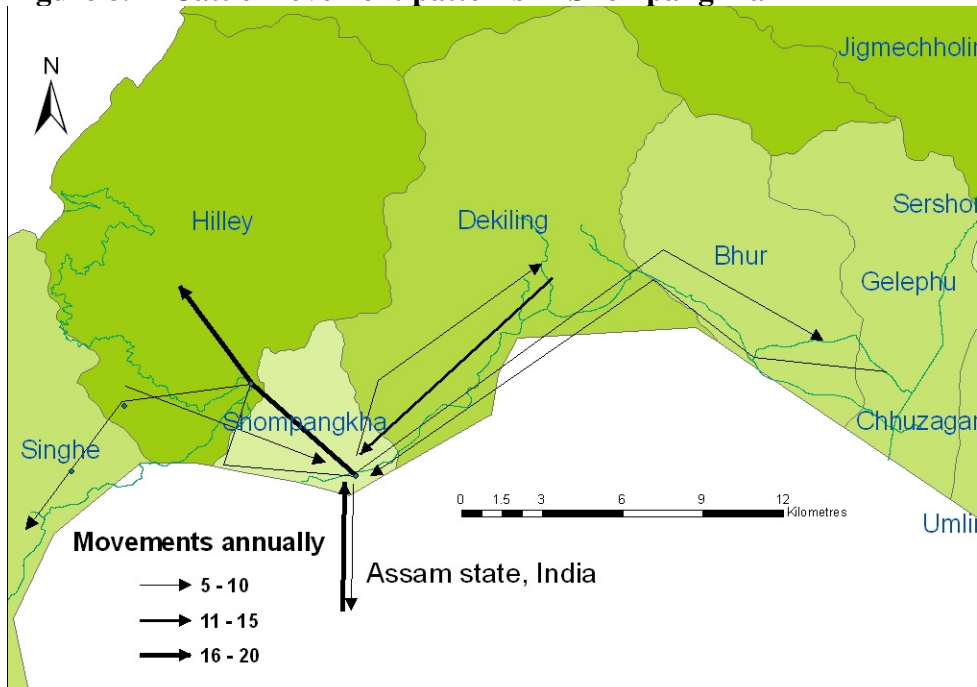
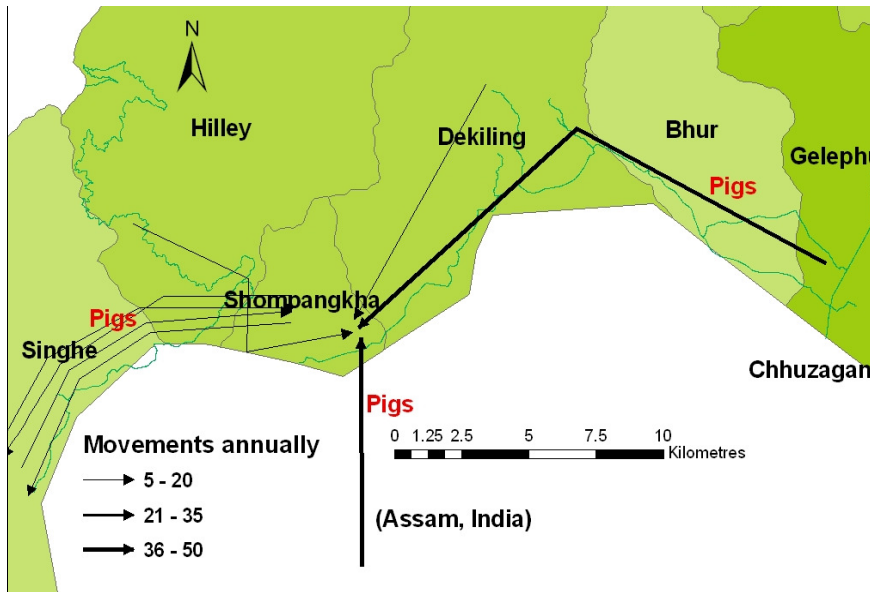


Figure 8.13 Movement patterns of goats and pigs between Shompangkha and other sub-districts and India.



In Chhuzagan, there were equal movements of cattle into and out of this sub-district and movements of cattle and pigs from India. In Umling and Sershong, there were more inward than outward movements for all species of livestock. In Dekiling and Bhur, there were more outward than inward movements of cattle and goats. There were no reports of cattle

being purchased from India (*Gup*, Bhur, personal communication). In Hilley, more inward movements of cattle and more outward movement of goats existed. The farmers in this sub-district also purchased cattle from India. In Singhe, more inward movements of cattle and goats existed, some of which originated from India. In Taraythang, only a few farmers owned livestock as this is a newly created sub-district. At the time of this study, farmers were not selling livestock. However, farmers purchased cattle and pigs from the neighbouring sub-district of Umling. There were no traders operating in this sub-district. Dovan sub-district doesn't have road connectivity and electricity but has one of the highest cattle population in Sarpang (Anonymous, 2009a). Dovan is one of the main sources of cattle and goats, which are purchased by the sub-traders and taken to the butchers in the towns for slaughter. There was limited inward movement originating from Jigmechhoeling.

8.3.5 Unofficial animal movements into Sarpang district

Approximately 500 cattle were reported to move across the border (Figure 8.14) between India and Gelephu sub-district, every month, for grazing purposes through various points such as Maokhola River, Tankey busty and Majigaon villages (*Gup*, Gelephu, personal communication). These cattle graze together with the cattle from Gelephu and then return to their villages in India in the evenings.

In Chhuzagang, there were two entry points, one originating from Dathgari village in Assam and following along the Maokhola River (*Gup*, Chhuzagang, personal communication). The other originated from Bagmara village and proceeded along the Taklai River (Figure 8.14). Approximately 10 cattle per month entered through these illegal routes and mixed with the cattle from this sub-district. Cattle from the neighbouring

Indian village of Bagmara entered through the illegal route and mixed with cattle from Umling sub-district (Figure 8.14).

There were four unofficial routes connecting Bhur sub-district with Tukrey and Dathgari villages in Assam. In Dekiling, there were three unofficial routes through which cattle from across the border mixed at grazing areas with the ones in the sub-district (Figure 8.15). One route passed along the Lewkhola stream, another one alongside the Teenbahgikhola, and the third route passed alongside the Dholkhola River.

In Shompangkha, live piglets were frequently traded through the three available unofficial routes. The first route passed alongside Akhaukhola, the second one through an old petrol station premise, and the third one through the Indo-Bhutan gate (Figure 8.15). Sometimes, cattle were also traded along this route.

Hilley sub-district had two unofficial trading routes. The first one passed alongside the Sarpangchu River and the second one along the Gurungchu River (Figure 8.15). Live piglets were purchased from India and brought along these routes where there is exchange or buying and selling of cattle between the Bhutanese and Indian farmers.

Singhe sub-district had two unofficial trading routes. Cattle from the Indian villages mixed with local cattle at the grazing grounds. The first route passed along the Bisty River and the second one passed through the Sisty River (Figure 8.15).

8.3.6 Livestock trading practices in Tsirang

Tsirang district, with an area of 638.3 square kilometres, and altitude ranging from 400 to 2000 m.a.s.l., is located in the south-central part of Bhutan (<http://www.tsirang.gov.bt/profile.php>, accessed on 30th October 2009). Around 58% of the total land area is under forest cover comprising mainly broadleaf and pine vegetation. The forest cover is also inclusive of the barren land which is used for grazing by animals.

The remaining 42% of the land is under agricultural cultivation. The district is comprised of 12 sub-districts and 65 villages. Rice, maize and millet are the major cereal crops grown while oranges, cardamom and vegetables are the principal cash crops. Mandarins constitute an important source of cash income for most of the farmers. Livestock rearing forms an integral part of the overall agricultural production system through provision of draught power and farm yard manure. A national highway connecting the southern border town of Sarpang to the capital city passes through this district. This district has a good network of roads and mule tracks and consequently most sub-districts and villages in the district are accessible by road. The livestock trading pattern was similar to Sarpang district. There was no organised livestock market in Tsirang for the sale of live animals. There was only one licensed animal trader/butcher in Tsirang who employed several sub-traders or middlemen to look for cattle in Tsirang, as well as other districts. He paid them a commission or fee of Nu 500 (US\$=11) per cattle sourced. These sub-traders were usually farmers who undertook the additional work of middlemen in their villages after the end of the agricultural season. Traders from other districts such as Thimphu, Wangdue Phodrang and Punakha also bought cattle from the sub-districts within Tsirang and took them to Gosarling slaughter house for slaughter and the meat was then taken back to the respective districts for sale. For goats, the sub-traders took live animals from Tsirang and sold them to other agents/butchers working in the northern districts of Thimphu, Wangdue Phodrang and Punakha. These agents/butchers slaughtered goats in the forest and then sold meat to customers in the towns.

The traders had been in the livestock business for a median of 3 years (range: 2-10). On average, a trader traded a minimum of 10.5 (range: 4-240) and a maximum of 20 (range: 6-250) cattle per year. The volume of livestock traded are detailed in Table 8.3.

Table 8.3 Median numbers (range) of animals traded annually by traders in Tsirang

Species	Category of traders	Minimum number traded (range)	Most likely number traded (range)	Maximum number traded (range)
Cattle	Full-time*	240	245	250
	Part-time	10 (4-45)	15 (5-50)	20 (6-60)
	Both	10.5 (4-240)	15 (5-245)	20 (6-250)
Goats [#]	Part-time	16 (7-25)	21.5 (13-30)	28 (16-40)
Pigs [#]	Part-time	14 (10-18)	25 (15-35)	45 (30-60)

* Only one full-time cattle trader in Tsirang; [#]No full time traders for goats and pigs in Tsirang

All (n=17) of the traders owned livestock. Most (16/17) traders walked their animals when sold to other farmers or traders. Most (11/17) also used trucks to transport their animals for sale. The majority of the middlemen held their purchased animals in their villages before sale and the butchers held the animals near the slaughter house. Animals were predominantly traded from September until April. Goats and pigs were also slaughtered in the villages with or without the approval of the regulatory authorities.

The average price for each species of animal traded in this district is given in Table 8.4.

Table 8.4 Median price in Ngultrums (range) per adult animal for each species in Tsirang district

Species	Minimum (range)	Most likely (range)	Maximum (range)
Cattle	5000 (4000-9000)	6000 (5000-10000)	6500 (5500-12000)
Goats	2350 (1000-2500)	2750 (1500-4000)	3250 (2500-5000)
Pigs	5000 (2500-6000)	5500 (4000-6500)	6500 (4500-8000)

Note: IUS \$ = 45 Nu at the time of the study (February-March 2010)

All of the traders (n=17) reported that the farmers wouldn't sell a sick animal to them. The majority of the traders reported hearing (14/17) about FMD and also could recognise

(13/17) some of the key clinical signs of FMD. The traders were well aware of the need to get official approval from the local livestock authorities at the sub-district and district level and also from the BAFRA before trading animals.

8.3.6.1 Cattle trading pathways in Tsirang

Cattle were the main species traded and the trading network was fairly well established. Cattle originated from most of the sub-districts and moved towards Gosarling sub-district where the slaughter house is located (Figures 8.16 and 8.17).

8.3.6.2 Goat and pig trading pathways in Tsirang

Trading of goats and pigs was minimal when compared to cattle. Goats, originating mainly from sub-districts such as Phuentenchu, Shemjong, Patala and Mendrelgang, were trucked to other districts such as Thimphu, Punakha, Wangdue Phodrang, Trongsa, and

Figure 8.16 Trading pathways in Tsirang for cattle originating from Patala, Phuentenchu, Tsirangtoe, Tsholingkhar and Shemjong sub-districts.

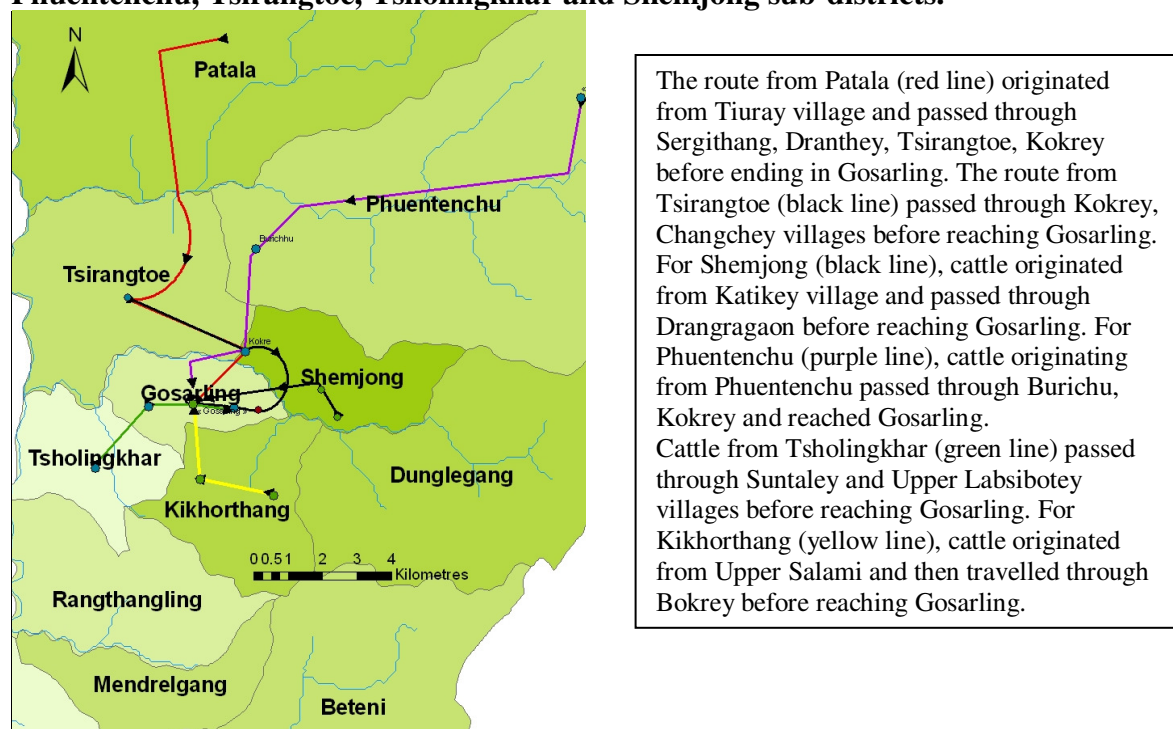


Figure 8.17 Trading pathways for cattle in Tsirang originating from Dunglegang, Beteni, Rangthangling, Mendrelgang and Barshong sub-districts.



Cattle originating from different villages in Rangthangling (red line) were walked or trucked along the Tsirang-Wangdue Phodrang highway through Kikhorthang until Gosarling. For Mendrelgang (blue line), cattle originating from various villages after reaching the road point were walked or trucked along the Mendrelgang-Damphu road to Gosarling. For Dunglegang (black line), cattle originated from Bichgaon, and passed through Salami, Bokrey before converging into Suntaley village of Gosarling. Cattle originating from Toesang and other villages passed through Barshong (purple line) and Phirphire villages before reaching Mendrelgang road point after which they were walked or trucked until Gosarling. For Beteni (yellow line), cattle originating from different villages were taken to

Zhemgang for slaughter and sometimes for breeding purposes. In most cases, goats were slaughtered in the villages and the meat was then transported to other districts for sale. Tsirang was also one of the main sources of fattened pigs. Owing to the lack of religious restrictions, farmers in this district reared pigs for fattening purposes and sold either live pigs or pork to traders who took these to other districts.

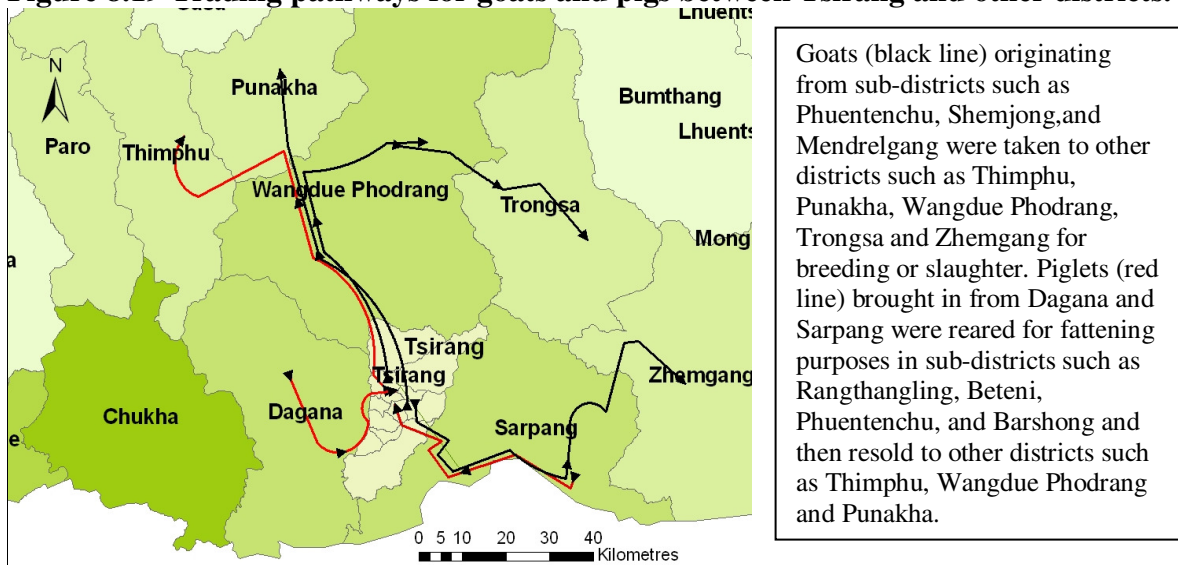
Local breeds of piglets were mainly brought from Dagana district especially from the villages of Sunkosh and Drukjeygang. The farmers bought the piglets from these villages directly from the sellers and walked or trucked them to their destination villages (Figure 8.18).

Figure 8.18 A farmer from Tsirang walking his piglet (bought from Dagana district) along the highway



The farmers also bought piglets from the local market in Damphu and walked them to their respective villages. The trading pathways for goats and pigs in Tsirang are outlined in Figure 8.19.

Figure 8.19 Trading pathways for goats and pigs between Tsirang and other districts.



8.3.7 Livestock movement patterns between the sub-districts

The general trading patterns were similar in all of the sub-districts in Tsirang. The farmers did not buy animals directly from India. The village traders operating in the sub-districts bought and sold cattle to the main trader in Gosarling. The farmers slaughtered goats and pigs in the villages for personal consumption and also for sale.

For Tsirangtoe, there were only outward movements for both cattle and goats destined for the neighbouring sub-districts. For pigs, there were only inward movements.

For Patala, there were more outward than inward movements of cattle. This sub-district shares its northern border with Wangdue Phodrang district where FMD is endemic. Some sub-traders also supplied live cattle to the main traders in other districts such as Wangdue Phodrang, Thimphu and Sarpang for slaughter.

In Shemjong, there were more outward than inward movements for all species. Farmers rarely purchased dairy cows from other districts because each sub-district had one Jersey bull for the breeding programme and there were enough Jersey cross-bred cows in the sub-district.

In Gosarling, there were more inward than outward movements of all species.

In Kikhorthang, there were both trading pathways as well as migratory pathways for cattle. Cattle migrated between this sub-district and Dunglegang and Beteni. There were more outward than inward movements for all species. For goats, there were only internal movements within the sub-district.

In Phuentsenchu, there were both inward and outward movements of cattle but only inward movements of pigs. Although Phuentsenchu shares a border with Dovan and Jigmechhoeling sub-districts of Sarpang district and Athang sub-district of Wangdue Phodrang, there was no intermixing of animals between these sub-districts

because of natural barriers (*Gup*, Phuentenchu, personal communication). The livestock traders took goats to other districts such as Wangdue Phodrang, Trongsa, Zhemgang, Thimphu and Punakha, for slaughter.

In Beteni, both inward and outward movements for all species existed. Cattle from other sub-districts migrated to this sub-district during winter for grazing. Local breeds of pigs were purchased from Drukjeygang and Sunkosh villages of Dagana district for breeding as well as for fattening purposes. Although Beteni shares borders with Nichula, Hilley and Singhe sub-districts of Sarpang, where FMD is endemic, there was no mixing of animals because of natural barriers of huge tracts of forests (*Gup*, Beteni, personal communication).

Movements of cattle and goats were restricted to Mendrelgang and Kikhorthang sub-districts only. Pigs were purchased from Drukjeygang and Sunkosh villages in the Dagana district for breeding and fattening purposes. The border between Barshong and the sub-districts of Drukjeygang and Trashiding in Dagana is separated by the Sunkosh River, therefore, animals from Dagana did not mix with the animals of this sub-district. In Mendrelgang, while there were more inward movements of cattle and goats, there were more outward movements of pigs.

In Rangthangling, there were more outward than inward movements of all species. The southern tip of this sub-district is separated from Dagana district by the Sunkosh River and therefore there was no mixing of cattle from Dagana and Rangthangling. Exotic breeds (Duroc, Saddleback) of pigs were bought from Gelephu in Sarpang, whereas local breeds were usually purchased from Sunkosh and Drukjeygang villages in Dagana.

In Dunglegang, there were only outward movements of cattle. Cattle were also taken to Bumthang district via Wangdue Phodrang and Trongsa for breeding purposes. Pigs originated from Rangthangling and Kikhorthang sub-districts. Cattle from Dunglegang

(Lalikharka village) migrated to Hilley sub-district in Sarpang and to Kikhorthang sub-district.

8.3.8 Comparative volume of livestock movements in Sarpang and Tsirang

More livestock were traded for both breeding (Table 8.5) and slaughter purposes (Tables 8.1 and 8.3) between the sub-districts in Sarpang than in Tsirang. There were more inward than outward movements of cattle and other species into the sub-districts of Sarpang than in Tsirang. In six of the 12 sub-districts in Sarpang there were movement of livestock from India through the unofficial entry points and mixing with livestock in these sub-districts. No animals were reported to have been purchased from India in the sub-districts of Tsirang and there were no illegal routes through which animals could enter into Tsirang from India or from the Sarpang district.

8.3.9 Livestock movement demographics from official data sources

Between 2007 and 2009, a total of 413 cattle and 892 goats moved out of Tsirang to various districts for the purpose of breeding or for slaughter. Most of the movements were towards Thimphu, Wangdue Phodrang and Punakha (Anonymous, 2010).

As per official records maintained at the BAFRA office in Tsirang (Anonymous, 2010), between 2007 and 2009 a total of 747 cattle were brought into Tsirang from Dagana and Chukha for slaughter in the Gosarling sub-district. The meat (beef) was subsequently sold in other districts.

8.3.10 Disease status in the study area

The status of FMD in each of the sub-districts in the study area is detailed in Table 8.6.

Table 8.5 Median (range) numbers of animals purchased or sold per month from each sub-district for breeding or rearing purposes in Sarpang and Tsirang

Sold or Purchased	Cattle	Goats	Pigs
SOLD (Sarpang)			
Minimum (range)	2 (0-15)	1 (0-6)	0 (0-10)
Most likely (range)	3 (0-20)	2 (0-8)	1 (0-14)
Maximum (range)	4.5 (0-35)	3 (0-10)	2 (0-15)
SOLD (Tsirang)			
Minimum (range)	1 (1-3)	1 (0-4)	1 (0-6)
Most likely (range)	2.5 (1-4)	2 (0-6)	1.5 (0-8)
Maximum (range)	3.5 (2-6)	3 (0-8)	2.5 (0-9)
PURCHASED (Sarpang)			
Minimum (range)	2.5 (1-10)	1 (0-5)	1 (0-6)
Most likely (range)	4.5 (2-35)	2 (0-7)	2 (0-8)
Maximum (range)	6 (3-50)	3 (0-8)	3 (0-9)
PURCHASED (Tsirang)			
Minimum (range)	1 (0-3)	1 (0-1)	1 (0-6)
Most likely (range)	2 (0-4)	1 (0-2)	2 (0-8)
Maximum (range)	3 (03-5)	1.5 (0-3)	3.5 (0-12)

(Source: Questionnaire interviews/focus discussions)

Table 8.6 Details of outbreaks of FMD in Sarpang and Tsirang districts (1996-2009)

Sub-districts	Years outbreaks reported	Last outbreak	Serotype(s)	Source
Sarpang district				
Jigmechhoeling	2007, 2009	2009	O	VIS
Shompangkha	2004	2004	O	VIS
Gelephu	1996, 1997, 1998, 2003, 2007	2007	O	VIS
Chhuzagang	1997, 2003, 2007	2007	O	VIS
Umling	1997, 1999, 2003, 2007	2007	O	VIS
Sershong	2003, 2007	2007	O	VIS
Dekiling	2002	2002	O	VIS
Bhur	Nil	Nil		VIS
Hilley	2003, 2004, 2007	2007	O	VIS
Singhe	1996, 2000, 2003	2003	O	VIS
Taraythang*	No information	No information		
Dovan	Nil	Nil		VIS
Tsirang district				
Tsirangtoe	Nil	Nil	-	VIS, FGD
Patala	Nil	Nil	-	VIS, FGD
Shemjong	Nil	Nil	-	VIS, FGD
Gosarling	Nil	Nil	-	VIS, FGD
Kikhorthang	Nil	Nil	-	VIS, FGD
Phuentenchu	Nil	Nil	-	VIS, FGD
Beteni	1998	1998		VIS, FGD
Tsholingkhar	Nil	Nil	-	VIS, FGD
Barshong	1998	1998		VIS, FGD
Mendrelgang	Nil	Nil	-	VIS, FGD
Rangthangling	Nil	Nil	-	VIS, FGD
Dunglegang	Nil	Nil	-	VIS, FGD

*Newly created sub-district in 2008; VIS: Veterinary information system; FGD: Focus group discussion with the heads of sub-districts and livestock extension officials in February-March 2010.

8.4 Discussion

Livestock trading was found to be largely unorganised, particularly for small ruminants and pigs. For cattle, because of the existence of slaughter houses in both districts, the trading system was fairly organised with a network of sub-traders involved in the purchase of cattle from the villages and supplying to the main traders for slaughter. For goats and pigs, it was either the sub-traders or the farmers who were involved in the trading of animals. As most of the sub-traders worked part-time without valid trade licenses, livestock trading occurred in a non-transparent form. This trading occurred in two forms: one for slaughter and the other for breeding and rearing purposes. The traders were found to be primarily involved in trading for slaughter purposes.

Livestock trading was found to be conducted more frequently in the dry season i.e. between October and December, and between February and April. The period between May and September (summer) was the least preferred for livestock trading due to the heat and rains which were not conducive for the transportation of animals. Therefore, in future, disease surveillance activities need to be intensified during the period of peak livestock movement activity.

More animals moved towards towns such as Gelephu and Shompangkha, in Sarpang than villages. Similarly, more cattle moved to Gosarling sub-district in Tsirang where the slaughter house is located. This indicates that the movement of cattle is driven by demand for meat by the urban population. This presents potential risks for the introduction of disease into the population unless strict quarantine and movement regulations are implemented. Since the slaughter houses cater only to cattle, ante-mortem and post-mortem inspection is restricted to this species only. In contrast, goats and pigs, and occasionally some cattle, were slaughtered in the villages of most sub-districts. In these circumstances, there was no inspection by the regulatory authorities and therefore such

practices pose potential risks to public health and may facilitate disease transmission. For instance illegal slaughter of infected pigs in the urban environment and feeding of uncooked swill was responsible for the persistence of infection in the Bicol region of the Philippines between January 1995 and October 1999 (Windsor *et al.*, 2011).

The volume of animals traded between the sub-districts, both for slaughter and breeding purposes were higher in Sarpang than in Tsirang. Overall, more animals moved into the sub-districts of Sarpang as compared with Tsirang. This presents the potential for disease introduction and spread (Christley *et al.*, 2005, Abila and Foreman, 2006) in Sarpang. In a questionnaire interview with the farmers (Chapter 5), approximately 15% (n=12/79) of the farmers in Sarpang reported buying animals from other districts whereas only 3% (3/88) of the farmers interviewed (Chapter 9) in Tsirang reported buying animals from other districts. Owing to the long and porous border with India, illegal trading of cattle and pigs was prevalent in those sub-districts of Sarpang bordering India. Some of the farmers in these areas also hired in or rented out their bullocks to Indian farmers. All the sub-districts that had illegal trading routes with the Indian villages had outbreaks of FMD within the 5-year period preceding this study (Table 8.6).

Although cattle were imported from other districts into Tsirang, the majority of these were for slaughter. Since there is only one highway connecting Tsirang with districts to the north, all animals entering Tsirang had to pass through police check points along the way and it is likely that animal consignments will be inspected by the BAFRA officials working at these check posts. However, the risks associated with introduction of FMDV into Tsirang through the movement of animals is very real given the fact that infected animals can excrete virus at least 24 hours before the development of clinical signs (Sutmoller *et al.*, 2003). Local breeds of pigs reared in Tsirang originated mainly from

Dagana where FMD is still endemic and therefore the unrestricted movement of live pigs poses a risk of introducing FMD into the susceptible population of Tsirang.

Most movements originating from villages were seldom subject to checks until the animals reached their destination at the slaughter houses. The checks conducted at the slaughter houses are not particularly beneficial given that these are “dead-end” movement pathways. It would be, however, crucial to monitor the movement of animals from the origin until they reach the slaughter house as there is a high risk of disease transmission during this period. Livestock moved to nearby sub-districts for breeding and rearing purposes were seldom checked by the regulatory agents and therefore potentially carried risks for disease transmission.

Given the sensitive nature of this study, the findings need to be considered with caution. Although the majority of the traders were willing to share information with the researcher, some were reluctant because of fear of reprisals from the authorities. This was due to the fact that most sub-traders were unlicensed and most interviews were conducted in the presence of the local veterinary authorities. However, the participants were briefed about the purpose of this research and the blinding techniques used to ensure that their names and identities would not be revealed in any reports or publications. The fact that collective opinions, of the *Gups*, *Mangmis*, *Tshogpas* and the livestock extension officer working at the sub-districts, were used for this study provides more confidence in the validity of the data. These government officials and farmer representatives have regular contact with the farmers for the day-to-day functioning of the sub-districts and therefore have good information about the farming activities and animal movement patterns prevalent in their respective sub-districts. Therefore, it is less likely that interviewee bias was present in this aspect of the study.

Given the limitations of funds and logistical challenges, the methodology used in this study was found to be effective in unravelling the movement patterns in the study area. In the absence of data on animal movements prior to the commencement of this study, this methodology was useful in collecting data based on the collective opinions of the community leaders.

In conclusion, the study showed that there was widespread movement of animals between the sub-districts for various purposes and at varying intensities. The unofficial movement of animals along the border areas could pose significant risks to the introduction and persistence of FMDV in Bhutan.

Recommendations

In order to implement cost-effective and risk-based approach to the control of FMD, it is essential to streamline the livestock trading system and understand the patterns of animal movements. Therefore, the following recommendations are proposed to further strengthen the control programme of FMD in Bhutan:

- **Establishment of livestock markets in the districts**

There is a need to establish at least one livestock market or auction yard in each district where farmers can buy and sell animals. Currently, owing to a lack of livestock markets, a lot of livestock trading occurs without the knowledge of the authorities and this can potentially result in spread of disease. If livestock markets are developed, it is expected that the traders would buy and sell animals through these locations and the BAFRA can inspect and monitor the movement of animals more effectively. It would also be possible to conduct routine surveillance, both serological and clinical, of important infectious diseases at these establishments. This potentially would also reduce the number of illegal movements of animals by the traders.

- **Establishment of more slaughter houses**

The study has shown that there is widespread slaughter of cattle, goats and pigs in the villages with little or no inspection by the regulatory authorities. This could, apart from creating risk for disease spread, pose serious health risks for the people. Only cattle are slaughtered at slaughter houses in Tsirang and Sarpang while small ruminants and pigs are slaughtered in the villages with no ante-mortem or post-mortem inspections. This could pose significant biosecurity risks for disease transmission.

- **Livestock Identification System (LIS)**

The key to effective management of animal movements is the ability to identify each animal in terms of its age, breed, gender, origin and location. A LIS would enable the regulatory authority to effectively monitor the movement of animals from birth until death. At the moment, Bhutan doesn't have a LIS. Although some of the exotic breeds of cattle in a few districts have been ear-tagged and identified, this information is not

entered into a database system to make it usable for tracing the movements of these animals.

Therefore, there is a need to establish an LIS similar to the ones used in other countries such as the National Livestock Identification System (NLIS) of Australia (<http://www.mla.com.au/Meat-safety-and-traceability/Livestock-identification/NLIS-database>; accessed on 23rd September 2010) or the National Animal Identification and Tracing (NAIT) (<http://www.maf.govt.nz/mafnet/animal-identification-and-tracing.htm>; accessed on 23rd September 2010) of New Zealand. An LIS could be used to identify all cattle in the country and enable monitoring of the movements.

- **Database on animal movement**

Data on the details of animal movements in the country are not appropriately recorded. For instance, although some data are available on the number of cattle imported through the entry points into Bhutan, there is no information on the destination of these imported animals and there is no system in place to track the movement of these animals. It is, therefore, difficult to know which districts the imported animals are destined for. Similarly, movements of animals from one district to another are rarely recorded or entered into a database. Most of the animal movements, especially those occurring between sub-districts, are similarly not recorded into a database.

To start with, although it may not be possible to identify all individual animals, it would be useful to have information on the numbers, species, source, destination and the purpose of movements. Animal movements occurring, at least between the districts, should be entered into this database system so that the overall movement patterns for each year can be reviewed. This can then be compared with the disease outbreak patterns. The movements occurring between the sub-districts should also be monitored

and entered into a database system by the respective livestock extension officers at the sub-district level. A database system similar to the ones used in Australia (NLIS), New Zealand (NAIT) or other developed countries should be initiated to monitor animal movements in the country.

- **Awareness programme**

Since the present system of livestock trading is expected to continue for some years, the best possible intervention from a disease control perspective would be to educate the livestock traders and farmers on various disease prevention and control measures. The traders should be made aware of the key clinical signs of infectious diseases, including FMD, and some basic biosecurity measures to adopt while trading animals. It is important to engage the private sector, especially the traders and butchers, into understanding the concepts of disease prevention if effective disease surveillance and control activities are to be achieved.

- **More research**

Studies such as this can only provide a “snapshot” of the animal movement patterns in the study area at a point in time. As livestock trading and animal movements are dynamic processes, it is essential to continue surveillance and research to understand the animal movement patterns on a continuous basis. Preferably, longitudinal studies need to be conducted, for at least 3 to 4 years, to unravel the complete livestock movement patterns in the country.

Although FMD is endemic in Bhutan, Tsirang district has been reportedly free from FMD since 1998. In the next chapter the results of serological and clinical surveillance are reported to validate this reported FMD-free status in the Tsirang district.

CHAPTER NINE

Serological and clinical surveillance to validate reported FMD-free status in Tsirang district of Bhutan

This Chapter has been published in a peer-reviewed journal:

Dukpa, K., Robertson, I.D. and Ellis, T.M. (2011) Clinical and serological surveillance studies to validate the reported foot-and-mouth disease free status in Tsirang district of Bhutan. *Preventive Veterinary Medicine*, In Press, doi:10.1016/j.prevetmed.2011.10.015

9.1 Introduction

In the FMD-endemic countries of South-East Asia efforts are being made to control and eventually eradicate FMD from the region by the year 2020 (Anonymous, 2007e). The OIE-led project - the South-East Asia and China Foot-and-mouth Disease control and eradication campaign (SEACFMD) uses the concept of progressive zoning (Edwards, 2004, Edwards and Abila, 2004) as one of the key elements for the programme. This approach is used given the limited resources both from within the participating countries as well as from donor countries (Abila, 2010) for the FMD control programme. Zoning is an approach aimed at identifying geographical areas of varying disease status within a country or region for the purpose of trade, disease control and eradication (Anonymous, 2007e). Zones are clearly defined areas to facilitate movements within zones, control movements between zones and to enable the control and eventual eradication of a disease. In the

SEACFMD member countries, zone status can include infected, control, eradication, free with vaccination, free without vaccination and buffer/surveillance zones. The aim is to progressively upgrade areas from infected zones to control zones, from control to eradication zones, from eradication to free zones with vaccination, and ultimately to free zones without vaccination (Anonymous, 2007e). Although FMD is endemic in most districts in Bhutan, the disease has not been reported in Tsirang district since 1998 as per the passive surveillance system (Anonymous, 2009b). The district shares a border with three other districts (Dagana, Wangdue Phodrang, Sarpang) where the disease is endemic (Anonymous, 2009b). Given the budgetary constraints for disease control activities, and the continued risk of incursion of new strains of FMDV through unregulated movement of animals across international borders, there is a need to initiate zoning approaches in Bhutan to control the disease progressively through focused and effective use of scarce resources. Initially, epidemiological studies need to be conducted to support the reported disease freedom status of Tsirang district before the initiation of zoning approaches.

Tsirang district is one of the more progressive districts in the country as far as livestock production is concerned. The district supplies good quality exotic cattle breeds for breeding purposes to other districts. The district also has one of the highest small ruminant populations in the country (Anonymous, 2009a) and supplies sheep and goats to other parts of the country for breeding and meat purposes. If the reported FMD-freedom status in this district can be confirmed by clinical and serological surveillance, there is a good opportunity to identify this district as a potential disease-free zone and progressively work towards expanding the zone in the future. This will pave the way for application of a progressive zoning approach in Bhutan as a means of achieving control and eradication of FMD in the future. Once identified as a FMD-free zone, this district can be one of the potential sources of livestock and livestock products for the country and for export.

Serological surveillance, in combination with clinical surveillance, is an accepted methodology (OIE, 2009a) to prove freedom from infection with FMD. A statistically valid surveillance programme targeting all susceptible species is required in a country to confirm freedom from FMDV circulation (OIE, 2009a). In countries where FMD vaccination is routinely undertaken, such as in Bhutan, NSP tests should be used to monitor the circulation of FMDV in the susceptible population (OIE, 2009a). Clinical surveillance for FMD can be done directly through close physical inspection of animals for clinical signs (OIE, 2009a) or indirectly through interviewing of livestock owners/keepers (Bronsvoort *et al.*, 2003). The OIE (2009a) clearly outlines the sampling procedures and methods to be used to follow up on any NSP-positive animals to confirm the absence of active infection in the population. Serological surveys have been used in other countries to demonstrate freedom from FMD (Picão-Gonçalves *et al.*, 2003, Isa, 2006).

In this study a combination of serological and clinical surveillance methods were used to validate the reported disease-free status of the district (as determined by the current passive surveillance system).

The specific objectives of this study were:

- To determine the presence of sub-clinically infected animals in the population by serological surveillance
- To validate the disease-free status of this district as reported by the passive disease surveillance system to provide confidence in the current disease surveillance system.

9.2 Materials and Methods

9.2.1 Study Area

A cross sectional study (first sampling) was conducted in Tsirang district from March 2009 until May 2009 following receipt of approval by the Animal Ethics Committee of Murdoch University (R2207/08). Repeat serological and clinical surveillance was conducted in November 2009 in all the NSP-positive herds/villages and all the positive animals, along with their herd-mates, were examined for any clinical lesions of FMD. For the randomized sampling during the first survey, the study was conducted in 20 villages of the 11 sub-districts in Tsirang district. The second sampling (November 2009) involved collection of samples from the villages (n=16) containing NSP positive animals on the first sampling and also from pigs. In the third round of sampling (February 2010), 20 villages located in high-risk areas were sampled. Sixteen villages which contained seropositive animals at the third sampling were resampled in August 2010.

9.2.2 Sampling strategy

For the purpose of this chapter villages were considered as the primary epidemiological units and the animals as secondary units.

9.2.2.1 First serological and clinical survey

For the first serological survey undertaken between March and May 2009, a two stage random sampling procedure was undertaken to select villages and animals for sampling. In the first stage, a list of all available villages was obtained (DLO, 2009) and then the required number of villages was selected by using simple random sampling without replacement (see Section 9.2.3 for sample size calculations). For selected villages, a list of all households owning livestock was made and then five to six households were selected by simple random sampling. From each household, four to five animals were selected also

by simple random sampling. The data on livestock owners in each village and livestock population in each herd was also obtained from the district livestock office.

Of the total from each village, at least 70% were cattle and the remaining 30% were small ruminants (sheep and goats) when available. This was based on the latest population figures for Tsirang (2007) where cattle (n=11727, 61.9%) were the main species reared followed by goats (n=4936, 26%), pigs (n=1382, 7.3%), sheep (n=723, 3.8%) and buffalo (n=184, 1%) (Anonymous, 2009a). Since not all herds contained all species, selection of small ruminants was done on an *ad hoc* basis based on the availability of the animals in a herd. All animals sampled, including their herd mates, were checked for clinical signs or lesions of FMD.

9.2.2.2 Second serological and clinical survey (follow-up to first survey)

All households that contained seropositive animals from the first sampling were revisited in November 2009 (second survey) and all seropositive animals, along with 1 to 4 herd mates were sampled. The animals were identified on the basis of their owner's name and the animal's name, age, sex and breed. These animals were also examined for the presence of any clinical signs or lesions of FMD and a detailed background history was collected using a pre-designed questionnaire (Appendix 5).

9.2.2.3 Targeted serological and clinical survey (third survey)

A purposive and targeted serological and clinical survey was undertaken in February 2010 in areas perceived to be at higher risk of FMD infection, namely villages located along the major roadways, and villages located close to the neighbouring FMD-endemic districts of Sarpang, Dagana and Wangdue Phodrang. All animals sampled, including their herd mates, were examined for the presence of clinical signs or lesions of FMD.

9.2.2.4 Fourth survey (follow-up to third survey)

All seropositive animals from the third sampling that were available, including some of their herd mates, were examined for lesions of FMD and sampled again in August 2010. The seropositive animals from the fourth sampling could not be revisited and sampled again owing to time and funding constraints.

9.2.3 Sample size

9.2.3.1 Sample size for the first and third serological surveys

To demonstrate freedom from disease in a large population, it is essential to calculate the sample size at two levels in order to account for clustering of diseases (Cameron and Baldock, 1998). Therefore, the design prevalence of FMD was accounted for at the village level and at the individual animal level while calculating the two stage samples.

First-stage sample (village)

Using FreeCalc Version 2 (Cameron and Baldock, 1998) a sample size of 20 villages needed to be sampled from the 65 available villages in order to detect the disease at a design prevalence of 20%. The village-level sensitivity was set at 95% (type 1 error for second-stage sampling set at 0.05), specificity at 99% (type 2 error for second-stage sampling set at 0.01), a minimum expected prevalence (MEP) of 20% among the villages, and the type 1 and type 2 errors for the entire survey were set at 0.05 and 0.05 to obtain the sample size of 20 villages. A minimum of two villages needed to be test positive in order to conclude that the district was not free from FMD.

When dealing with surveillance of infectious diseases it is acceptable to assume that at least 2% of the herds (or villages in this case) and 5% of the animals within infected herds (or villages) will have been infected (Greiner and Dekker, 2005). In Bhutan a village (consisting of a number of herds) can be considered as a “herd” since most of the animals in the village are managed under an extensive system whereby the animals owned by

different farmers have frequent contact during grazing and watering. The animals in a village are typically managed under similar management conditions and would therefore be exposed to similar risk factors (Chapters Five and Seven). Therefore, for the purpose of this chapter, a village was considered as a “herd”.

The MEP at the village-level was kept at 20% in view of the fact that villages are mostly contiguous to each other and in the event of the occurrence of a highly contagious disease such as FMD a higher proportion of the villages would be infected. Since the biosecurity procedures followed in the villages are either very rudimentary or non-existent, it is not unusual to find FMD spreading rapidly between villages in a short period of time (Chapters 3 and 4).

Second-stage sample (animals)

With an assumed expected prevalence of 25% in the infected village, diagnostic test sensitivity and specificity of 97% and 99% respectively, an average population of 245 susceptible animals per village (Anonymous, 2009a), and Type I (α) and Type II (β) errors set at 0.05 and 0.01, a total of 23 animals needed to be randomly sampled from each village. The total number of animals to be sampled from each selected village depended on the livestock population in the individual villages (Cameron, 1999) and was determined on a case-by-case basis. A village was classed as positive if more than two animals were found to be test-positive.

9.2.3.2 Sample size for the second serological (follow-up) and clinical surveillance

For the second sampling (November 2009) in seropositive villages, four to five animals were sampled from herds that were seropositive on the first survey. Owing to the lower and scattered population of pigs within the district, convenience sampling was used for

sampling the pigs. Sampling from pigs was undertaken whenever a farmer owned pigs along with cattle.

9.2.3.3 Sample size for the targeted (third) survey

The methodology for determining the sample size for the third sampling was the same as that for the first survey.

9.2.4 Sample collection and laboratory tests

All sera were collected, coded, sealed and preserved at -20°C in a freezer (Chapter 6).

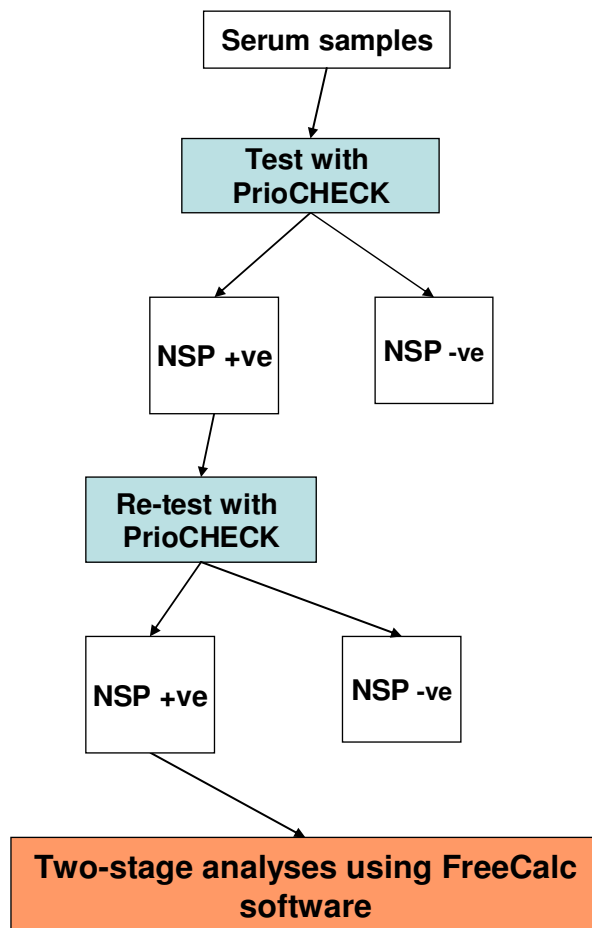
NSP ELISA tests were used to detect antibodies against FMDV in the sampled animals. A commercial NSP kit, PrioCHECK[®] FMDV NS, from Prionics AG (Switzerland) was used initially. The test kit was used as per the manufacturer's instructions. Test sera with PI of 50% or more were classed as positives and PIs below 50% were classed as negatives. All positive sera were re-tested using the same kit to reduce the number of false positives (Paton *et al.*, 2006). Those sera which were positive on both occasions of testing with the PrioCHECK[®] FMDV NS (PrioCHECK) were retested with another NSP test, CHEKIT-FMD-3ABC bo-ov (CHEKIT) (IDEXX, Bern, Switzerland). The kits were used as per the test protocol and procedures previously described for PrioCHECK (Sørensen *et al.*, 1998, Sørensen *et al.*, 2005) and CHEKIT (Bruderer *et al.*, 2004). Those sera which tested positive to both the PrioCHECK and CHEKIT were sent to the Australian Animal Health Laboratory (AAHL), Geelong, for confirmation using a c-ELISA developed by that laboratory (Foord *et al.*, 2007).

9.2.4.1 Testing protocol

The two-stage analyses (animals and villages) for demonstration of freedom was based on the test results of the PrioCHECK only (Figure 9.1). For the first and third survey, all PrioCHECK-positives were retested using the PrioCHECK and only those samples which

gave positive results on both occasions were classed as positive. The results of this were then entered into FreeCalc (Cameron and Baldock, 1998) to confirm freedom from infection. However, additional tests were undertaken on the NSP positives (PrioCHECK) using CHEKIT and c-ELISA, in series, to demonstrate that these were all likely false and not true positives (Figure 9.2).

Figure 9.1 Testing protocol adopted for the two-stage analyses to demonstrate freedom from infection

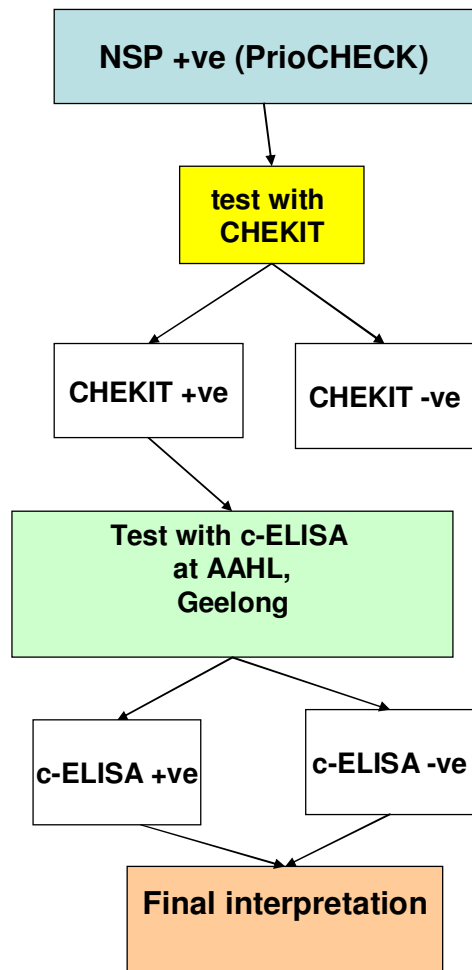


9.2.5 Questionnaire

Pre-designed questionnaires (Approved by the Murdoch University Human Ethics Committee – no. 2008/257) were developed and administered to the selected farmers

(Appendices 1 and 5). The questionnaire was written in English since the enumerators were well versed in English and pre-tested and applied as detailed in Chapter Five.

Figure 9.2 Testing protocol adopted to confirm the PrioCHECK positives using CHEKIT and the c-ELISA



9.2.6 Data analysis

Data were managed and statistical analyses performed as described in Chapters 5 and 6.

9.2.6.1 Interpretation of the analyses of the FreeCalc programme for disease freedom

Demonstration of freedom from disease was performed using the FreeCalc programme in Survey Toolbox (Cameron and Baldock, 1998, Cameron, 1999). The data were analysed in two stages. Firstly, the data from each village were analysed to provide a village level

result, indicating whether the village was positive or negative. The population size for each village; the correct Type I and II error levels selected for the second stage of sampling; the survey sample size; the number of seropositives for each village; the test's sensitivity and specificity; and the MEP were entered into the FreeCalc software for the analyses.

When all villages had been analysed separately, the population of villages was analysed in the second stage to demonstrate that the whole population was free from disease. The data entered were the herd test sensitivity and specificity, the total number of available villages, number of villages tested, Type I and II error levels for the whole survey and the MEP between the villages.

The results were displayed in terms of the probabilities of the null and alternative hypothesis (Cameron, 1999). The probability of observing this many reactors or fewer, if the population was diseased at a level equal to or greater than the specified prevalence, is the probability of the null hypothesis. If this probability is small, it is very unlikely that the population is diseased at a given confidence level. Conversely, if the probability is large, there is not enough evidence to claim that the population is free from disease. If the probability of the alternative hypothesis is small, then it is unlikely that the population is free from disease. Conversely, if it is large, then it is likely that the population is free from disease (Cameron, 1999).

9.3 Results

9.3.1 Serological results from the first survey

9.3.1.1 Background profile of the study population

A total of 536 animals were sampled from 20 villages spread across 11 sub-districts. Cattle (72%) constituted the bulk of the animals sampled followed by goats (27%) and sheep (1%).

9.3.1.2 Seroprevalence for the first sampling

Based on the PrioCHECK NSP test, the overall test seroprevalence of FMD at the animal-level for all species in Tsirang was 3% (16/536; 95% CI: 1.7, 4.8). The seropositive animals were distributed in 13 villages of which 12 had one seropositive each and one village had four seropositives. Only one of 144 goats (0.7%, 95% CI: 0.0, 3.8) and none of the sheep (0.0%, 95% CI: 0.0, 60.2) were seropositive. A total of 15 of 388 cattle (3.8%, 95%CI: 2.2, 6.3) tested positive to NSP antibodies. There were no significant differences between the prevalence in cattle (3.9%, 95% CI: 2.2, 6.3) and in small ruminants (0.7%, 95% CI: 0.0, 3.7). The number of seropositives in a village was determined to identify any clustering of cases (Table 9.1).

Table 9.1 Distribution of seropositives from the first sampling

Sub-districts	No. of villages positive/tested	No. of villages having seropositives			
		1	2	3	4
Dunglegang	2/2	2	0	0	0
Gosarling	2/2	2	0	0	0
Kikhorthang	1/2	1	0	0	0
Mendrelgang	4/4	2	1	0	1
Rangthangling	2/3	2	0	0	0
Tsholingkhar	1/1	1	0	0	0
*Tsirangtoe	1/2	1	0	0	0
Total	13/16	11	1	0	1

**Tsirangtoe village had only one seropositive goat*

9.3.1.3 Disease freedom status using the FreeCalc programme for the first survey

The demonstration of freedom from disease was done in a two-stage analysis (Cameron and Baldock, 1998). In the first stage, the test results of the individual villages were analysed using the FreeCalc computer programme as displayed in Table 9.2.

Table 9.2 Serological status of the villages from the first survey

Sub-districts	Villages	No. of NSP positive animals/sampled	P-value for null hypothesis	P-value for alternative hypothesis	Confidence of detecting FMD at the design prevalence (%)	*Result for village
Dunglegang	Khorsaney	1/27	0.003	0.126	99.60	N
Dunglegang	Bichgaon A	1/23	0.009	0.206	99.03	N
Beteni	Beteni	0/25	0.0005	1.000	99.94	N
Tsholingkhar	Allanchey	1/26	0.003	0.229	99.68	N
Mendrelgang	Pemashong	4/25	0.194	0.000	80.56	P
Mendrelgang	Mendrelgang	1/27	0.001	0.237	99.83	N
Mendrelgang	Riserboo A	1/27	0.003	0.237	99.68	N
Mendrelgang	Tashipang	1/25	0.005	0.117	99.47	N
Patala	Sergithang	0/26	0.0004	1.000	99.96	N
Phuentenchu	Manithang	0/32	0.00005	1.000	99.99	N
Rangthangling	Dhajay	1/30	0.001	0.260	99.84	N
Rangthangling	Sunkosh	1/25	0.004	0.222	99.59	N
Rangthangling	Neveray	0/26	0.0003	1.000	99.96	N
Shemjong	Kokrey	0/28	0.0002	1.000	99.97	N
Tsirangtoe	Tsirangtoe	1/30	0.001	0.260	99.89	N
Tsirangtoe	Damtsang	0/31	0.00008	1.000	99.99	N
Gosarling	Upper Labsibotey	1/27	0.002	0.237	99.72	N
Gosarling	Gairigang A	1/26	0.003	0.229	99.67	N
Kikhorthang	Upper Salami	1/26	0.004	0.229	99.53	N
Kikhorthang	Lower Bokrey	0/24	0.0007	1.000	99.92	N

*Results for village based on PrioCHECK NSP test. N=Negative, P=Positive

Except for Pemashong all villages were considered negative for FMD. For these disease-free villages, the confidence of detecting FMD, if it was present at the design prevalence (village-level sensitivity), was >99%.

Second stage analyses using the FreeCalc programme

The probability of observing 1 test positive or fewer in a sample of 20 villages from a population with a disease prevalence of 20% was 0.045 (Null hypothesis) and the probability of observing 1 or more reactors in a sample of 20 villages from a disease free population was 0.182 (Alternative hypothesis). Therefore, these results are adequate to reject the null hypothesis and conclude that the population is free from disease (at the MEP of 20%) at the 95.53% confidence level. That is, we are >95% confident that the survey would have detected FMD if 20% or more of the villages in the district were infected.

9.3.1.4 Results of the testing of seropositives with CHEKIT and c-ELISA (AAHL, Geelong)

The available PrioCHECK NSP-positive sera (13 of the 16 in total) were retested using CHEKIT (Bommeli) in Bhutan and a c-ELISA at AAHL. The results are summarised in Table 9.3.

Table 9.3 Retesting results of NSP positives with CHEKIT and c-ELISA

Total samples retested	CHEKIT			c-ELISA		
	No. of positives	No. of negatives	No. of suspects	No. of positives	No. of negatives	No. of inconclusive
13	4	4	5	3	8	2

Note: Out of 16 positives, only 13 were retested as there were insufficient sera for the remaining three samples

Only three samples initially testing positive to the PrioCHECK were again positive to both the CHEKIT and c-ELISA (Table 9.4 and Figure 9.3). All these NSP-positive animals did not show any clinical signs or lesions suggestive of FMD and these animals had all been born and raised within their current village.

Table 9.4 Animals testing positive to PrioCHECK, CHEKIT and c-ELISA (sampled in March 2009)

Sub-districts	Villages	Species	Age (years)	Breed	Date of last vaccination
Mendrelgang	Pemashong	Bovine	4	Local	January 2008
Mendrelgang	Tashipang	Bovine	15	Jersey cross	January 2008
Kikhorthang	Upper Salami	Bovine	16	Local	June 2008

9.3.2 Serological results for the second survey from the NSP positive herds

All 16 animals that tested positive to the PrioCHECK from the first sampling were visited and resampled along with some of their herd mates. A total of 83 animals including 73 cattle and 10 goats were sampled eight months after the initial sampling from the NSP-positive villages (Table 9.5). Not all herd mates of the seropositive animals could be sampled and therefore animals (n=41) from other neighbouring herds were sampled, as well.

Figure 9.3 Location of the three seropositive villages in Tsirang district following retesting with CHEKIT and c-ELISA from the first survey

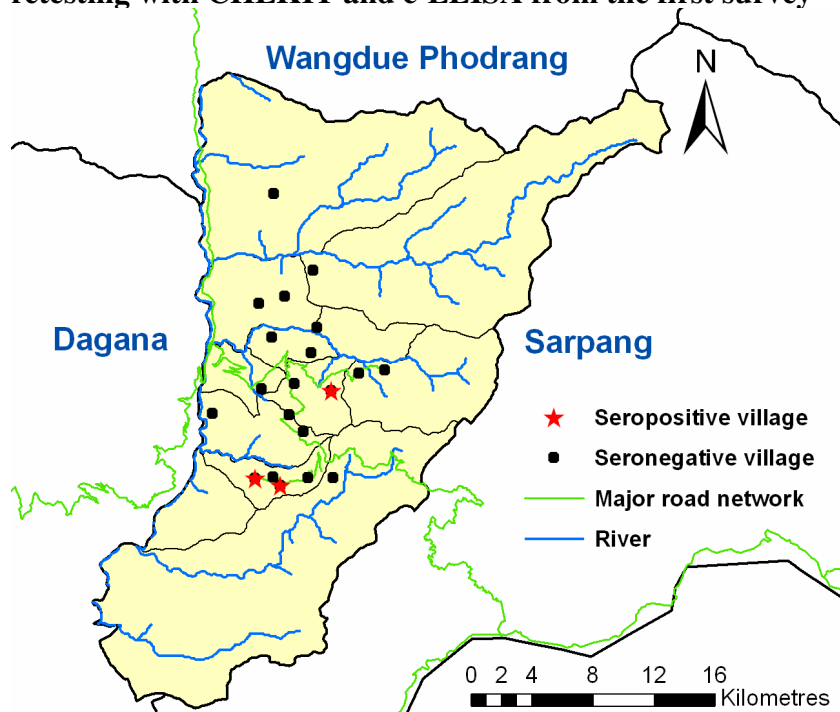


Table 9.5 Sampling details from the seropositive villages in November 2009

Sub-districts	Villages	No. of NSP positives in first sampling		No. of animals resampled in November 2009	
		Bovine	Caprine	Bovine	Caprine
Dunglegang	Khorsaney	1	0	6	0
Dunglegang	Bichgaon A	1	0	4	1
Gosarling	Upper Labsibotey	1	0	4	0
Gosarling	Gairigang A	1	0	6	0
Kikhorthang	Upper Salami	1	0	4	1
Mendrelgang	Riserboo A	1	0	1	0
Mendrelgang	Tashipang	1	0	2	0
Mendrelgang	Mendrelgang	1	0	5	0
Mendrelgang	Pemashong	4	0	24	4
Rangthangling	Dhajay	1	0	4	2
Rangthangling	Sunkosh	1	0	5	0
Tsholingkhar	Allanchey	1	0	5	0
Tsirangtoe	Tsirangtoe	0	1	3	2
Total		15	1	73	10

Nine of the 83 samples (10.8%, 95% CI: 5.1, 19.6) were positive to the PrioCHECK and these originated from eight villages in seven sub-districts. Of these, eight were cattle and a goat from Pemashong village in Mendrelgang sub-district.

Only one village (Sunkosh) had two seropositive animals. Of the 16 seropositive animals from the first sampling (March 2009) resampled, five were positive to both PrioCHECK and CHEKIT on the second round (November 2009) of testing eight months after the initial sampling. Of these, three were positive to the c-ELISA. However, no herd mates of these three seropositive animals were positive to either the PrioCHECK or CHEKIT (Table 9.6).

Table 9.6 Results for the seropositives from the first sampling and their herd mates during the second round of sampling in November 2009

Sub-districts	Villages	No. of NSP positives in first sampling	No. of herd mates sampled in November 2009	Were animals seropositive during first survey again positive on repeat sampling?	No. of herd mates testing positive to both tests in series
Dunglegang	Khorsaney	1	4	No	0
Dunglegang	Bichgaon A	1	4	No	0
Gosarling	Upper Labsibotey	1	3	No	0
Gosarling	Gairigang A	1	5	Yes	0
Kikhorthang	Upper Salami	1	4	Yes	0
Mendrelgang	Riserboo A	1	0	Yes	NHS
Mendrelgang	Tashipang	1	0	Yes	NHS
Mendrelgang	Mendrelgang	1	4	No	0
Mendrelgang	Pemashong	4	16	No	0
Rangthangling	Dhajay	1	0	No	NHS
Rangthangling	Sunkosh	1	4	No	0
Tsholingkhar	Allanchey	1	4	Yes	0
Tsirangtoe*	Tsirangtoe	1	1	No	0
Total		16	#49	5	0

NHS = no herd mates sampled due to unavailability of animals in the herd

**Caprine species, all others are bovine; #The total differs from that in Table 9.5 because not all seropositive animals had herd mates*

9.3.2.1 Results for the sampling from pigs

A total of 48 pigs were sampled from six villages located in six sub-districts during the second round of sampling. Since there were very few pigs reared in the village, and since these were scattered, targeted sampling was adopted based on the availability of the animals. All tested negative (0.0%, 95% CI: 0.0, 7.4)

9.3.3 Serological results for the third survey – targeted sampling

A total of 543 animals including cattle, goats, sheep and pigs were sampled from 20 villages spread across 7 sub-districts in the third sampling. Cattle (75%, n=408) constituted the bulk of the animals sampled followed by goats (20%, n=108), pigs (4%, n=21) and sheep (1%, n=6).

9.3.3.1 Seropositivity for the third survey

Nineteen of the 543 animals originating from 16 of the 20 villages (Table 9.14) were positive to the PrioCHECK, producing an overall (all species) test seroprevalence of 3.5% (95% CI: 2.1, 5.4). The seroprevalence in cattle was 4.6% (19/408; 95% CI: 2.8, 7.2). None of the goats (0/108; 0.0%, 95% CI: 0.0, 3.4), pigs (0/21; 0.0%, 95% CI: 0.0, 16.1) or sheep (0/6; 0.0%, 95% CI: 0.0, 45.9) were positive. Thirteen of the seropositive villages had only one seropositive animal and three villages had two seropositive animals.

9.3.3.2 Disease freedom study using the FreeCalc programme

In the first stage analyses, all villages (“herds”) sampled were found to be test-negative for FMD at the given level of confidence for the specified design prevalence of 25% (Table 9.7).

In the second stage analysis, the probability of observing 0 reactors in a sample of 20 villages (“herds”) from a population with a disease prevalence of 20% was 0.005 (Null hypothesis) and the probability of observing 0 or more reactors in a sample of 20 villages from a disease free population was 1.0 (Alternative hypothesis). Therefore, these results were adequate to reject the null hypothesis and conclude that the population was free from disease (at the MEP of 20%) at the 99.46% confidence level. That is, we are >99% confident that the survey would have detected FMD if 20% or more of the villages in the district were infected.

Table 9.7 Serological status of the villages from the targeted sampling

Sub-district	Villages	No. of positive animals/ sampled	P-value for null hypothesis	P-value for alternative hypothesis	Confidence of detecting FMD at the design prevalence (%)	*Results for village
Beteni	Beteni	1/20	0.023	0.095	97.67	N
Beteni	Bhulkey	1/20	0.024	0.095	97.62	N
Beteni	Thangray	1/25	0.006	0.117	99.34	N
Tsholingkhar	Harpeypani	1/27	0.002	0.126	99.71	N
Tsholingkhar	Lower Tsholingkhar	1/30	0.0001	0.139	99.85	N
Tsholingkhar	Tintaley	1/29	0.0009	0.135	99.91	N
Mendrelgang	Pemashong	1/17	0.045	0.082	95.43	N
Mendrelgang	Mendrelgang	1/23	0.006	0.108	99.32	N
Mendrelgang	Riserboo A	1/25	0.006	0.117	99.29	N
Mendrelgang	Tashipang	1/18	0.035	0.086	96.48	N
Rangthangling	Dhajay	0/27	0.0003	1.000	99.96	N
Rangthangling	Neveray	0/26	0.0004	1.000	99.96	N
Gosarling	Lower Labsibotey	1/27	0.003	0.126	99.68	N
Gosarling	Upper Suntolay	1/29	0.005	0.117	99.46	N
Kikhorthang	Lower Salami	0/26	0.0006	1.00	99.94	N
Kikhorthang	Upper Salami	1/25	0.006	0.117	99.33	N
Kikhorthang	Lower Bokrey	0/25	0.0006	1.000	99.94	N
Kikhorthang	Upper Bokrey	2/48	0.0000	0.020	99.99	N
Patala	Burichu	2/45	0.0000	0.025	99.99	N
Patala	Dovan Rilangthang	2/31	0.006	0.010	99.37	N

*Results for village based on NSP test. N=Negative, P=Positive

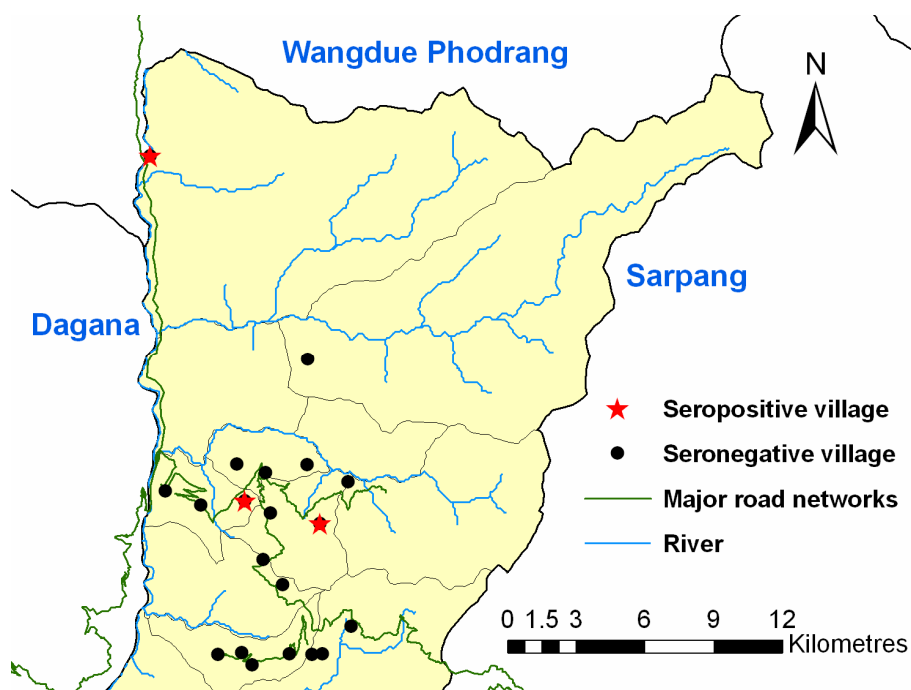
9.3.3.3 Results of the testing of seropositives with the CHEKIT and c-ELISA

When the 19 positive samples were retested with the CHEKIT, 10 tested positive, 2 were classified as suspects and the remaining 7 were negative. When these 12 positive/suspect samples were tested at AAHL, Geelong, only 3 tested positive (Table 9.8 and Figure 9.4).

Table 9.8 NSP positive animals that tested positive to PrioCHECK, CHEKIT and c-ELISA (February 2010)

Sub-districts	Villages	Species	Age (years)	Breed	Date of last vaccination
Patala	Rilangthang	Bovine	2.5	Local	December 2009
Tsholingkhar	Harpeypani	Bovine	6	Local	December 2009
Kikhorthang	Upper Salami	Bovine	3	Jersey cross	December 2009

Figure 9.4 Location of the three seropositive villages following retesting with CHEKIT and c-ELISA from the third survey



9.3.3.4 Serological results for the repeat sampling (fourth survey) of the NSP positives and herd mates from the targeted survey

Of 19 seropositive animals from the third sampling, only 15 could be resampled in August 2010 at the fourth survey since the other four had either died (n=2), were sold (n=1), or could not be traced (n=1). A total of 65 animals including 61 cattle and 4 goats, were sampled from the villages containing NSP-positive animals and the sera tested with the CHEKIT (Table 9.9).

A total of 46 herd mates and four other animals originating from neighbouring herds were sampled. Of the 15 seropositive animals from the third sampling, only one animal tested positive to the CHEKIT during the fourth round of sampling conducted after six months (Table 9.9). Nine of the 46 herd mates (19.6%, 95% CI: 9.4, 33.9) were also positive on this sampling. When the 10 seropositives to CHEKIT (Table 9.9) were retested with the c-ELISA, only one bovine (6.6%, 95% CI: 0.2, 31.9), originating from Rilangthang village, tested positive.

9.3.4 Results of the first questionnaire survey and clinical surveillance

9.3.4.1 Background of study population

A total of 99 farmers were interviewed from 20 villages spread across 11 sub-districts of Tsirang during the first survey in March 2009.

On average, each respondent had lived for 33 years in the village (range: 2-70 years). The main source of income was agriculture (76.7%) with the remaining 23.3% generated from livestock. The median area of land owned per household was 1.94 hectares (range: 0 – 10.5 ha).

Table 9.9 Results for the seropositives and their herd mates from the third sampling resampled again in August 2010 (CHEKIT only)

Sub-districts	Villages/ Herds	*No. of NSP positives in third sampling	No. of animals sampled including the herd mates	Seropositives from third sampling again testing positive to CHEKIT on fourth sampling?	No. of herd mates testing positive from fourth sampling
Beteni	Beteni	1	3	No	0
Beteni	Bhulkey	1	4	No	0
Beteni	Thangray	1	3	No	0
Kikhorthang	Upper Bokray	2	6	NAS	1
Kikhorthang	Upper Salami	1	3	No	1
Patala	Rilangthang	2	8	Yes	3
Patala	Burichu Dovan	2	7	No	0
Gosarling	Upper Suntolay	1	3	No	1
Gosarling	Lower Labsibotey	1	4	NAS	1
Mendrelgang	Riserboo A	1	5	No	1
Mendrelgang	Tashipang	1	2	NAS	0
Mendrelgang	Pemashong	1	4	No	1
Mendrelgang	Mendrelgang	1	2	No	
Tsholingkhar	Lower Tsholingkhar	1	3	No	0
Tsholingkhar	Tintaley	1	4	No	0
Tsholingkhar	Harpeypani	1	4	No	0
	Total	19	65	1	9

NAS = No animals sampled (either sold, died or untraceable); * *PrioCHECK*

9.3.4.2 Livestock production system

Farmers in Tsirang reared multiple species of livestock including cattle, buffalo, sheep, goats, pigs and poultry. Cattle (61.9%, 623/1007) were the most populous FMD-susceptible species in the surveyed villages followed by goats (30%, 302/1007), pigs (6.7%, 68/1007), buffalo (0.8%, 8/1007) and sheep (0.6%, 6/1007).

Cattle were reared mainly for production of farm yard manure (36.4%, 36/99), followed by dairy production (33.3%, 33/99) and draught purposes (31.3%, 31/99). However, this was not exclusive and farmers reared cattle for multiple purposes.

9.3.4.3 Farmer-reported prevalence of FMD

Of the 99 farmers interviewed, only 40 could recognise the clinical signs of FMD from a picture of lesions. However, when the name of the disease was explained in their local language, 72.7% (72/99) could remember some of the key clinical signs and lesions of the disease. Approximately half (50%, 50/99) of the respondents could remember some disease control measures to be undertaken in the event of an outbreak of FMD.

None of the farmers reported seeing FMD in their animals within the 5-year period preceding the survey. A few farmers (10.1%) reported having seen FMD in their herds more than 15 to 20 years preceding this survey.

9.3.4.4 Vaccination profile

The majority of the farmers (68.7%, 68/99) reported that their cattle had been vaccinated against FMD during the 5-years preceding the survey. Of these, 47.1% (32/68) and 44.1% (30/68) reported that their cattle were vaccinated twice and once a year, respectively. Few (2.9% - 2/68) farmers reported having had their cattle vaccinated once every 2-3 years whereas the rest were unsure how frequently their cattle had been vaccinated.

9.3.4.5 Animal movement pattern in Tsirang

Approximately three-quarters (75.8%, 75/99) of the farmers in Tsirang sent their animals for grazing, of which forest grazing accounted for 77.3% (58/75) followed by grazing on communal land (21.3%, 16/75) and paddy fields (20%, 15/75). None of the farmers who owned sheep and goats allowed their small ruminants to graze together with cattle. Of those farmers who purchased animals, 60.2% (53/88) had purchased them from other villages from the same district (Tsirang) whereas only 3.4% (3/88) had purchased animals from another district. Sixty-eight percent (60/88) of farmers reported purchasing animals from within the same village. These were not exclusive and some farmers purchased animals from multiple sources.

9.3.4.6 Clinical surveillance

All the animals were inspected for clinical lesions of FMD in the mouth and feet and also for evidence of hoof deformity which may have been associated with a history of FMD. None of the animals had lesions or signs that were indicative of FMD.

9.3.5 Results of the second clinical surveillance and questionnaire survey (NSP-positive herds from first sampling)

9.3.5.1 Prevalence of FMD

No respondents reported having seen clinical signs of FMD in their herds within the 5-year period preceding this survey. No seropositive animals or their herd mates had lesions or signs suggestive of FMD.

9.3.5.2 Management of animals

None of the owners of the seropositive animals reported buying cattle from other districts or from India within the 5-years preceding this survey.

9.3.6 Results of the third clinical surveillance and questionnaire survey

9.3.6.1 Study population profile

A total of 34 farmers were interviewed from 20 villages spread across 7 sub-districts during the third (targeted) survey in February 2010.

9.3.6.2 Farmer-reported FMD prevalence

Fifteen respondents could recognise FMD when a picture depicting key clinical signs of the disease was shown to them. However, when the name of FMD was explained in their local language, all could name some of the key clinical signs of the disease. No respondents reported seeing clinical signs or lesions similar to that of FMD in their herds or villages within the 5-year period preceding this survey. A few respondents (5/34) recalled outbreaks of FMD in their villages approximately 20 to 25 years ago.

9.3.6.3 Clinical surveillance

None of the animals examined had lesions or signs suggestive of FMD.

9.3.6.4 Management

Twenty-six respondents (76%) reported replacing cattle with ones they had bred themselves whereas 21 respondents (62%) reported purchasing replacement animals from other herds in their village. Only one respondent (3%) reported replacing cattle through the purchase of animals from other districts and none purchased cattle from India during the 5-years preceding this study.

9.4 Discussion

The study detected a very low prevalence of NSP-antibodies distributed across multiple villages. These findings are strongly suggestive of the absence of active infection in the FMD-susceptible population of Tsirang district. The seropositives observed were likely to

be false positives because of the following considerations: The distribution of the seropositive animals, during all periods of sampling failed to indicate clustering of cases in the villages (“herds”). For instance, the seropositives from the first and third (targeted) surveys were distributed among 13 of 20 and 16 of 20 villages sampled, respectively. In a large population where animals are separated into herds (or villages in this case), diseases tend to cluster (Cameron and Baldock, 1998) because of the uneven distribution of the disease-causing agents throughout the population. Had there been active infection circulating in the seropositive villages, given the highly contagious nature of FMD, it would have been expected to see clustering of cases (seropositives) in these villages. The fact that retesting from the seropositive villages failed to detect ‘clustered’ seropositives is highly indicative of the absence of active viral circulation in the study population. This was further supported by the findings of the two-stage probability sampling analysis using the hypergeometric exact probability formula. Although vaccination is used routinely in the area, the FMD vaccine, like many other vaccines, do not induce sterilizing immunity and may therefore allow replication of virus when vaccinated animals are exposed to infection (Doel, 2003). The FMD vaccines are also very unstable outside the range of 2-8°C (Kitching *et al.*, 2007), and given the scattered location of the herds and villages in Bhutan, effective application may be difficult (Chapter 4). Therefore, in the event of incursion of FMDV into a susceptible population, it is expected that animals would become infected and show clinical signs in spite of the population being vaccinated (Chapter 3).

The anti-3ABC antibodies can be detected in infected animals up to 665 days (Moonen *et al.*, 2004b) after infection. Had there been active infection circulating in the seropositive villages, then repeat sampling from the seropositive animals and their herd mates should have detected a significant amount of seropositivity. Only three of 16 animals from the first survey and one of 15 cattle from the third survey tested positive on repeat sampling

after eight and six months, respectively. None of the herd mates of the seropositive animals from the first survey tested positive when resampled.

The serological tests employed in this study consisted of two commercial and one in-house NSP tests. Although, these tests have been evaluated under various conditions, none of these tests has a perfect sensitivity and specificity (Bronsvooort *et al.*, 2004c, Sørensen *et al.*, 2005, Brocchi *et al.*, 2006, Kittelberger *et al.*, 2008). Consequently, even in countries free of FMD and where vaccination is not done, such as in New Zealand (Kittelberger *et al.*, 2008), false positives can occur.

In this study, the randomised sampling produced an overall seroprevalence of 3% (95% CI: 1.7, 4.8) which is within the expected range (2%) given the test's imperfect specificity (Brocchi *et al.*, 2006). Similarly, the targeted sampling produced an overall seroprevalence of 3.5% (95% CI: 2.1, 5.4). Given the test's imperfect specificity, these are likely to be false positives as none of the seropositive animals had a history of lesions or clinical signs of FMD (questionnaire interviews and focussed group discussion). In a serological surveillance to demonstrate freedom from infection in the Myanmar area of the Malaysia-Thailand-Myanmar zone, Oo (2010) also reported NSP seropositives (using PrioCHECK) in cattle from areas that had no evidence of clinical FMD for 10 to 15 years. These were also thought to be false positives given the test's imperfect specificity. In a structured two-stage seroprevalence study to demonstrate absence of active FMD infection in the Bicol region of the Philippines using LPBE (Windsor *et al.*, 2011), about 5% and 25% of the barangays (villages) had clusters of seropositive pigs and cattle, respectively, although no clinical cases of FMD were reported during the study period (March and September 1999). Although, some of the seropositives were thought to be as a result of unapparent recent FMD infection, most of these were concluded as false positives arising out of the imperfect specificity of the LPBE (98.5%).

Multiply vaccinated animals tend to develop detectable levels of antibodies to the NSPs depending on the level of these proteins in the vaccines (Clavijo *et al.*, 2004, Lee *et al.*, 2006). Although limited field studies (Chapter 6) indicate absence of NSP residues in the vaccines used in Bhutan, this has not been confirmed using a larger sample size and for a longer duration. Therefore, false positives arising out of NSP residues in the vaccines cannot be completely ruled out.

Only one goat tested positive (first survey). Had there been active FMDV circulating in the population, it would be expected that the small ruminants would also be infected given the extensive system of farming (Chapter 6). Since the small ruminants are rarely vaccinated against FMD, they could act as sentinel animals and could indicate circulation of active infection in the population as reported in other FMD-endemic areas with similar management conditions (Balinda *et al.*, 2009).

The PrioCHECK was used for screening and initial retesting of seropositives and the CHEKIT and c-ELISA were used as confirmatory tests for the seropositives. This testing regime of sequential NSP serology was found to be useful in reducing the number of false positives and clarifying the infection status of the herds. Similar testing approaches have been proposed for substantiating freedom from FMDV infection after emergency vaccination in previously disease-free countries of Europe (Paton *et al.*, 2006). When the seropositive samples were retested with the other two tests in series, the specificity improved and consequently the number of “false” positives declined dramatically.

All of the seropositive animals were revisited and sampled along with herd mates, as required by the OIE code (OIE, 2009b). On interviewing the owners of the seropositive herds, village headmen and livestock officers (Chapter 8) there were no reports of clinical signs similar to FMD in the herds within the 5-year preceding this study. Because of the close association with their animals, livestock owners generally have good knowledge

about the clinical and epidemiological features of FMD (Rufael *et al.*, 2008). Further, given the fact that FMD produces characteristic clinical signs, especially in cattle (Davies, 2002, Kitching, 2002b), any recent outbreaks would have been easily noticed and reported by the farmers to the local veterinary authorities.

Although the serological and clinical surveillance results support the belief that Tsirang district is free from FMD infection, the study is not without drawbacks. The limitations of this study stems from the fact that the design prevalence chosen for demonstration of disease-freedom was quite high (25% for within-herd and 20% for between-herd prevalence). This was chosen based on the fact that FMD is a highly contagious disease and given the extensive system of livestock farming and poor biosecurity practices in Tsirang and other districts (Chapters 3 and 4), it is expected that a large number of animals would be infected within a short period of time if disease was introduced (Chapter 4). Therefore, the design prevalence chosen was based on the disease's epidemiology (Cameron, 1999) and the livestock management system adopted in the study area.

Virus isolation and molecular tests (PCRs) could have been conducted on samples, such as oesophago-pharyngeal fluids, to confirm the infection/carrier status of the seropositive animals. However, owing to logistical and cost constraints and with the absence of facilities for virus isolation and molecular studies in Bhutan, these could not be performed. There is good potential to maintain the disease-free status in the future given the existence of natural barriers between Tsirang and the neighbouring FMD-endemic districts (Chapter 8). For instance, the Sunkosh River separates Tsirang from Dagana district and animals have to travel via bridges to move between the districts. Similarly, huge tracts of forests and steep cliffs separate the northern borders of Tsirang from Wangdue Phodrang. The borders between Tsirang and Sarpang are similarly filled with large tracts of tropical forests where there is no mixing of animals. However, to continue to maintain this status,

surveillance needs to be undertaken at least biannually in high risk areas, such as the entry points (check posts) into Tsirang, to identify any sub-clinical infections circulating in the population. In order to make up for the imperfect specificity of the NSP tests, all seropositive animals should be followed-up with virological tests such as virus isolation and PCR. There is also a need to create awareness about FMD amongst the farmers so that they can recognise clinical signs of FMD should an outbreak occur in the future. They should also be trained or made aware of the basic biosecurity measures to be undertaken if an outbreak of disease occurs. The whole of Tsirang district can now be designated as a control zone and accordingly modified FMD control measures applied. Vaccination can be restricted to the high risk areas such as in settlements along the roads and in areas bordering the three other FMD-endemic districts. Continuous surveillance should be undertaken in the villages and sub-districts bordering Tsirang. The bordering areas of Sarpang, Dagana and Wangdue Phodrang districts should now be designated as buffer zones and compulsory vaccination of all FMD-susceptible species should be done at least bi-annually and movements of cattle from these districts to Tsirang should be strictly regulated.

In conclusion, based on the results obtained through serological and clinical surveillance studies over a period of one-and-half years, it can be concluded that Tsirang district was free from FMD at the time of this study. However, sustained surveillance activities need to be undertaken to maintain this status. The study has paved the way for initiation of zoning approaches to the progressive control of FMD in Bhutan.

Vaccination has been one of the main tools to prevent and control FMD in Bhutan. So far, no information is available on the immune response and field efficacy of the current FMD vaccines used in the country. The next chapter reports the immune responses in cattle vaccinated against FMD under field conditions in Bhutan.

Foot-and-mouth disease vaccine induced immune status in cattle in Bhutan.

10.1 Introduction

Vaccination, either prophylactic or post-outbreak, is one of the main methods to control FMD in Bhutan (Anonymous, 2005). Commercially available trivalent vaccines (O, A, Asia 1), formulated with either an oil-adjuvant (Raksha Ovac[®]) or an alum-adjuvant (Raksha[®]), produced by Indian Immunologicals, Hyderabad, India have been used in Bhutan for many years (Anonymous, 2008a). Intervet India Pvt. Ltd. based in Pune, India, also manufactures a trivalent FMD vaccine containing the same serotypes and this vaccine has also been used in Bhutan, although in much lesser quantities and frequencies than the one from Indian Immunologicals. The sub-types for each serotype used in the vaccine include O *Manisa*, A₂₂, and Asia 1 *Shamir*. Vaccination is undertaken at least twice annually in the districts bordering India whereas the interior districts usually vaccinate once a year. However, the vaccination coverage and frequency can vary between herds, villages and sub-districts depending on the availability of vaccine, financial support to conduct vaccination, and cooperation of the livestock owners. Although a control programme is in place, there has been little success in reducing the incidence of FMD, owing to many factors including lack of information on the efficacy of the FMD vaccine and vaccination programme in the field (Anonymous, 2005, Dukpa *et al.*, 2011). Although FMD is endemic to many districts in Bhutan, Tsirang district is reportedly free from FMD

infection and has been since 1998 (Chapter 9). Therefore, a study was conducted to compare the FMD antibody status of vaccinated populations of cattle in FMD-endemic districts and an FMD-free district (Tsirang) so as to assess the effectiveness of the vaccination programme.

10.2 Materials and methods

10.2.1 Study area and design

The sera collected as part of the seroprevalence study in the endemic districts (Chapter 6) and disease freedom study in Tsirang (Chapter 9) were used in this study. Sera were randomly selected from the NSP negative sera originating from vaccinated herds that had no history of outbreaks of FMD (Chapters 5, 6, and 9) within a 5-year period preceding the survey.

10.2.2 Laboratory tests

The sera were analysed using the LPBE (Hamblin *et al.*, 1986a, Hamblin *et al.*, 1986b) at AAHL with slight modification to the test procedures as detailed below. The LPBE is a serotype-specific test that detects antibodies developed against the structural proteins of FMDV following vaccination or infection (OIE, 2008). The test is based upon specific blocking of the FMDV antigen in liquid phase by antibodies in the test serum sample. An LPBE kit procured from the WRLFMD in Pirbright was used.

Briefly, ELISA plates (NUNC Maxisorp) were coated with 50µl per well of rabbit antibody specific for A, O or Asia 1 serotypes diluted in coating buffer (1:5000 dilution). The ELISA plates were incubated for 1hr at 37°C on a plate rocker and then placed overnight at 4°C.

Using a transfer round bottom plate, the appropriate positive control and test sera were diluted four-fold down the plate starting at an initial dilution of 1 in 20 (finishing with 60µl

of solution per well). The negative control sera were tested at a 1 in 20 dilution in duplicate. A dilution of antigen (1 in 100) in liquid phase diluent of the relevant serotype or subtype was added to the transfer plate (60µl per well) to all wells except the background OD controls which received 60µl of diluent only. The transfer plates were incubated overnight at 4°C. Therefore, all sera were tested at a starting dilution of 1 in 40 because a titre of less than 1 in 40 is considered to be negative (Hamblin *et al.*, 1986b, OIE, 2008).

After overnight incubation, the ELISA plates were washed with PBSABC, and 50µl per well of diluted sample from the relevant transfer plate was added in duplicate to the corresponding wells on the ELISA plate. The ELISA plates were incubated at 37°C for 60 minutes on a plate shaker. Following incubation, the ELISA plates were washed with PBSABC, and 50µl per well of the appropriate dilution of homologous guinea pig detection antiserum was added to the plate. The plates were incubated at 37°C for 30 minutes on a plate shaker. After incubation, the ELISA plates were washed with PBSABC and 50µl per well of Rabbit anti-guinea pig immunoglobulins conjugated to horseradish peroxidase (1:2000 dilution) was added. The plates were placed at 37°C for 30 minutes on a plate shaker. Following incubation, the ELISA plates were washed with PBSABC and 50µl per well of commercial TMB substrate (Sigma) was added to each well. The plates were developed at room temperature for 10 minutes and then 50µl per well of stop solution (1 in 18 dilution of concentrated Sulphuric acid in water) was added to each well. The ELISA plates were read at 450nm on a spectrophotometer linked to a computer by blanking on a background well and reading the inhibition with OD (optical density) max wells as reference wells. A positive result was recorded when the sera reduced the maximum OD (OD of antigen control) by 50%. This is calculated by: $100 - (100 \times (\text{OD test} / \text{OD max}))$

serum mean/OD max mean)). Linear interpolation between serum dilution levels (as log scale values) was used to estimate titres at the 50% maximum OD level.

10.2.3 Data analysis

Descriptive and statistical analyses were performed as described in previous Chapters. Serum antibody titres were expressed as the logarithm (base 10) of the reciprocal of the highest serum dilution which produced a minimum of 50% inhibition. The titres for the positives ($\geq 1:40$) were all log transformed (base 10) before analysis. For the negatives (titres $< 1:40$), a fixed titre of 1:9 was assumed since the next higher dilution in the series would be 1 in 10. Therefore a fixed log transformed value of 0.954 was assigned to all samples that had titres less than 1 in 40 (negatives). Parametric tests such as the Student's t-test for independent samples and the one-way analysis of variance (ANOVA) were used to compare the mean titres between the districts after the data were checked for normality (Pallant, 2005). Correlation between age and antibody response was checked using the Pearson bivariate correlation coefficient (Pallant, 2005).

10.3 Results

10.3.1 Sampling profile

A total of 499 bovine sera from 75 villages from five districts were tested (Table 10.1). The months in which cattle in each district were last vaccinated against FMD using the trivalent FMD vaccine are summarised in Table 10.2.

Table 10.1 Summary of sampling done in the study area for the immune study

Districts	No. of sub-districts sampled	No. of villages sampled	Total sera tested (% of total)
Chukha	6	14	98 (19.6)
Sarpang	5	10	91 (18.2)
Trashigang	7	13	84 (16.8)
Trongsa	4	17	117 (23.5)
Tsirang	11	21	109 (21.9)
Total	33	75	499

Table 10.2 Dates of vaccination in the study area for the immune study

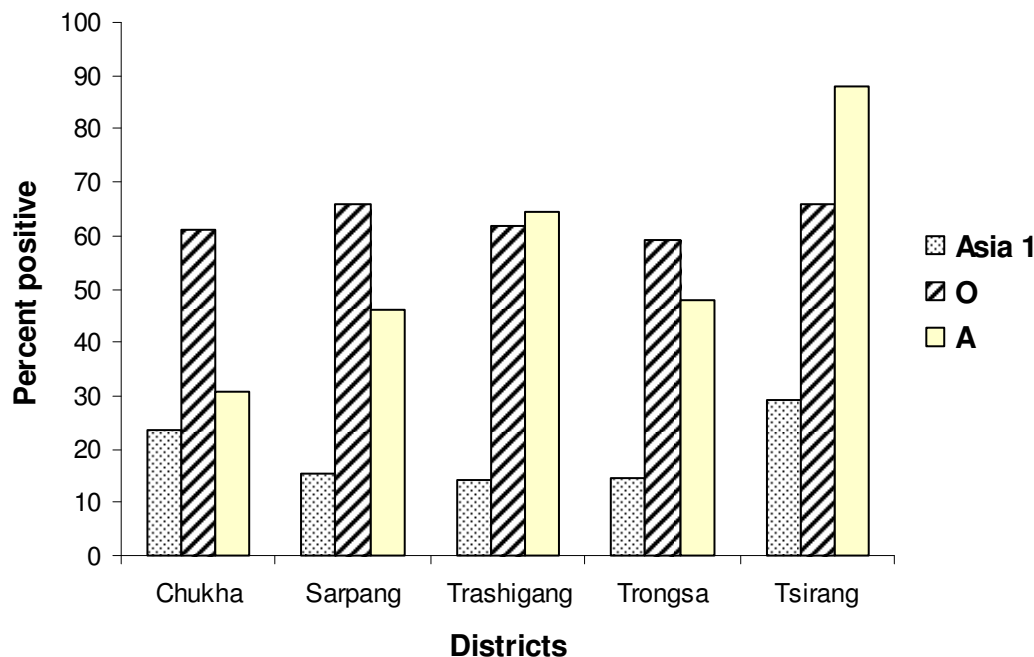
Districts	Months/year last vaccinated	Sampling date
Chukha	September, October 2008	April/May 2009
Sarpang	January, February, March 2008	March/April 2009
Trashigang	July, September, October 2008	April 2009
Trongsa	October-December 2008, January 2009	April 2009
Tsirang	June-July 2008	March 2009
	January-February 2009	

10.3.2 Overall serotype positivity

Overall, antibody to serotype O was the most prevalent (62.7%, 95% CI: 58.3, 67.0; 313/499). The next most prevalent serotype was serotype A (55.7%, 95% CI: 51.2, 60.1; 278/499) and Asia 1 (19.6%, 95% CI: 20.3, 28.9; 98/401). However, in Tsirang district, antibody to serotype A was more prevalent than to the other serotypes and in Trashigang the prevalence of antibody to serotype O and A was nearly the same (Figure 10.1). For Asia 1 serotype, there were significant differences ($P=0.018$, $\chi^2=11.9$, $df=4$) in the seropositivity between the districts with Tsirang district (29.4%, 95% CI: 21, 38.8; 32/109) recording the highest seropositivity and Trashigang (14.3%, 95% CI: 7.6, 23.6; 12/84) the

lowest. The seropositivity towards O serotype was similar in all districts. However, for serotype A, there were significant differences ($P < 0.0001$, $\chi^2 = 80.1$, $df = 4$) with Tsirang (88.1%, 95% CI: 80.5, 93.5; 96/109) recording the highest seropositivity and Chukha (30.6%, 95% CI: 21.7, 40.7; 30/98) the lowest.

Figure 10.1 Distribution of antibodies to different FMDV serotypes



10.3.3 Seropositivity between FMD-endemic and FMD-free districts

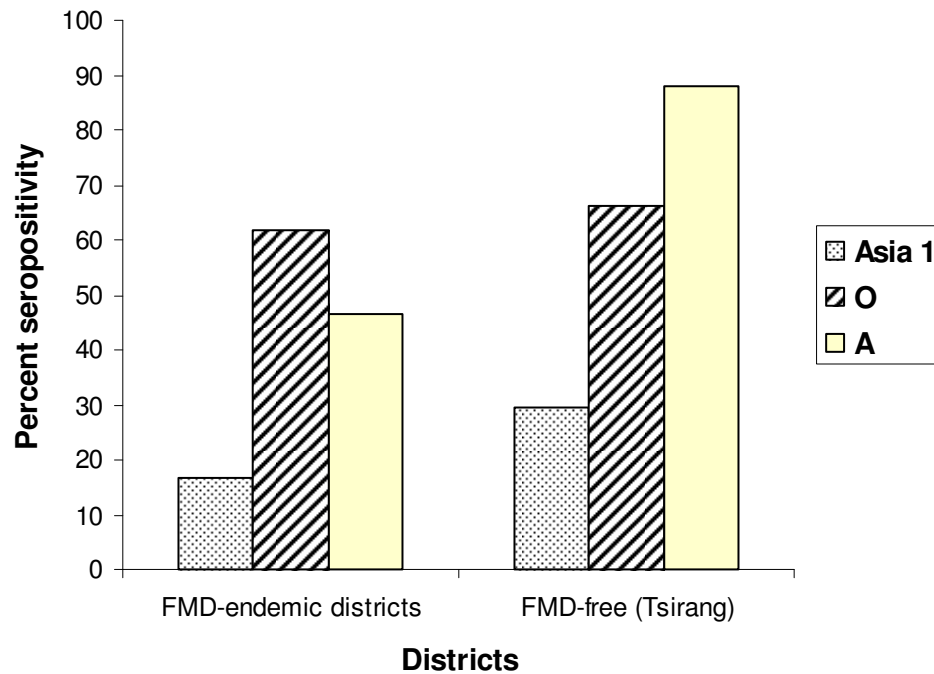
Tsirang district had a significantly higher seroprevalence to serotypes A ($P < 0.0001$, $\chi^2 = 50.2$, $df = 1$) and Asia 1 ($P = 0.009$, $\chi^2 = 6.9$, $df = 1$) than the FMD-endemic districts. However, the seropositivity to serotype O was similar between the two categories of districts (Figure 10.2).

10.3.4 Magnitude of immune response

The level of immune response in the vaccinated population was checked by assessing the antibody titres for each serotype. The geometric mean titre (GMT) for each serotype in each district is displayed in Figure 10.3. Overall, the GMT for serotype O (52.5 ± 1.07

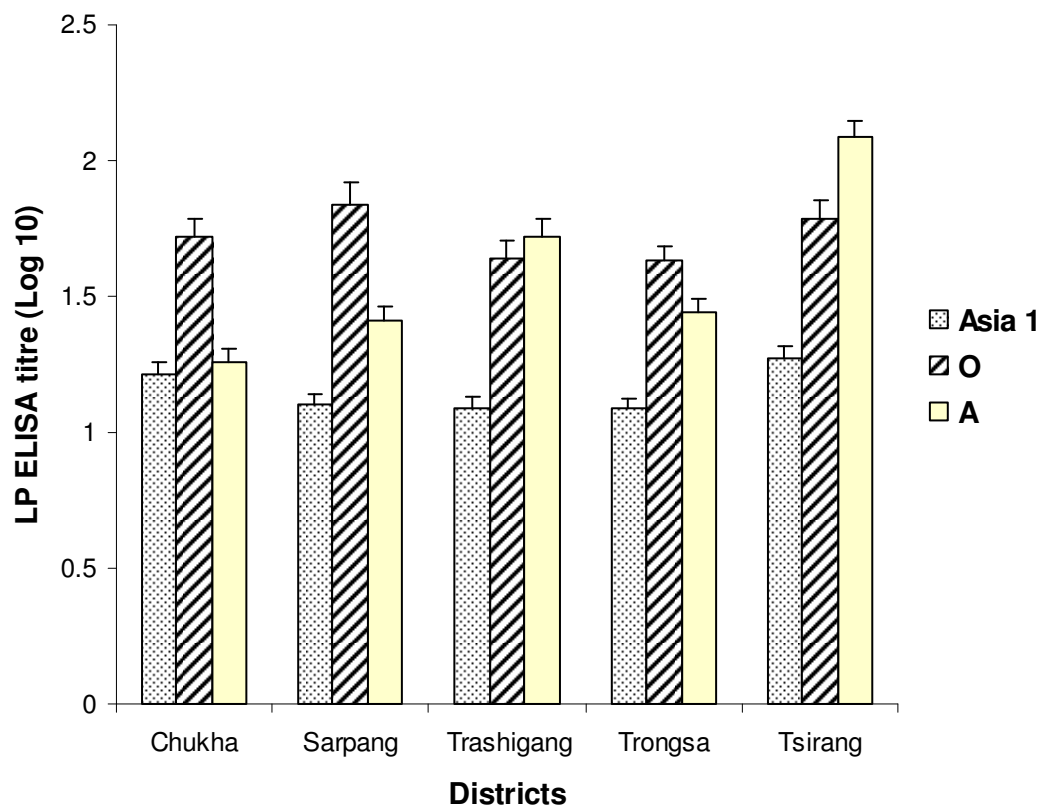
SEM) was the highest followed by A (38.0 ± 1.07 SEM) and Asia 1 (14.5 ± 1.04 SEM). There were significant differences in the mean titres between the districts for Asia 1 (ANOVA, $P=0.006$, $F=3.67$, $df=4$) and A ($P<0.0001$, $F=37.0$, $df=4$) serotypes.

Figure 10.2 Distribution of serotypes in FMD-endemic and FMD-free districts



For Asia 1, Tsirang had significantly higher titres than Sarpang, Trashigang and Trongsa whereas for serotype A, Tsirang had significantly higher titres than all other four FMD-endemic districts.

Figure 10.3 Distribution of the geometric mean log titre for the three serotypes



Note: the error bar indicates the standard error of the mean for each serotype

When the districts were grouped into FMD-endemic and FMD-free districts, cattle from Tsirang district had significantly ($P=0.008$, $t=-2.67$, $df=144.6$) higher GMT (18.6 ± 1.12 SEM) than did cattle from the endemic districts (13.5 ± 1.05 SEM) for Asia 1 serotype. This was also true for serotype A where Tsirang district had a significantly ($P<0.0001$, $t=-10.2$, $df=166.7$) higher mean titre (123.0 ± 1.14 SEM) than did the endemic districts (28.2 ± 1.07 SEM). However, the antibody response to O serotype was similar between endemic and disease free districts.

10.3.5 Correlation between age and antibody response

There were significant positive correlations between age and antibody titre for all three serotypes: Asia 1 ($r=0.24$, $P<0.0001$), O ($r=0.23$, $P<0.0001$) and A ($r=0.16$, $P<0.0001$).

Thus older animals were more likely to be seropositive.

10.4 Discussion

The study detected significant variation in the seroprevalence to the three FMDV serotypes in the study area. While antibodies to serotype O were the most prevalent in the endemic districts, antibodies to serotype A were the most prevalent in Tsirang district. Similar observations of differing immune responses to the three serotypes have been reported in studies conducted with the same vaccine in India as used in this study (Singh *et al.*, 2008a) or a quadrivalent (O, A, Asia 1 and C) vaccine (Chitravel *et al.*, 1997) also manufactured in India. This differing immune response could be a reflection of the varying antigen payload for the three serotypes used in the vaccine (Patil *et al.*, 2002) although no information was available on the amount of viral antigen used in the vaccine formulation from the manufacturer. The high seropositivity to O serotype in the endemic districts as compared to the other two serotypes could also be possibly due to sub-clinical infections in the vaccinated population which may not have been apparent to the farmers or which may not have been detected by the NSP test given the test's imperfect sensitivity. Serotype O has been the only serotype involved in outbreaks of FMD in Bhutan after 2003 (Dukpa *et al.*, 2011) which is consistent with the distribution of FMDV serotypes in the neighbouring countries of India (Verma *et al.*, 2008) and Nepal (http://www.wrlfmd.org/fmd_genotyping/asia/nep.htm accessed on 25th October 2010). The mean titre of serotype O was the highest followed by A and Asia 1. Except for antibodies to serotype A in Tsirang (mean log titre=2.09), all other serotypes had antibody titres below log 2.0 (1:100 titre) which is usually considered to be the protective titre in

vaccinated cattle (Hamblin *et al.*, 1987, Periolo *et al.*, 1993). Given that a trivalent vaccine containing serotypes O, A and Asia 1 had been used to vaccinate these cattle 6 to 12 months prior to this study (Anonymous, 2008a), the low level of antibody response, especially in the FMD-endemic districts, could be due to waning vaccine-induced immunity as the last vaccination conducted in these districts was more than 6-months ago. Alternatively, this could be due to poor vaccination coverage or an ineffective vaccination campaign. Similar observations of a low protective immunity against the serotypes O (only 32.7% at ≥ 2.1 mean log titre), A (39.8%) and Asia 1 (60.2%) were observed even after three rounds of vaccination using the same vaccine (Singh *et al.*, 2008a). However these authors could not determine the reasons for the poor immune response.

The FMD vaccines, especially vaccines with an aqueous-based adjuvant, are known to confer immunity up to 6 months only (Woolhouse *et al.*, 1996). In contrast the oil-based FMD vaccines can confer longer immunity, up to one year (Domenech *et al.*, 2010). However, several factors, including host factors, antigenic spectrum coverage of the strains used, cold-chain maintenance of the vaccines, and actual delivery mechanisms of the vaccine, can limit the effectiveness of vaccination (Garland, 1999, Doel, 2003).

The findings indicate that a relatively high proportion of cattle would be susceptible to infection should FMDV gain entry into these populations as none of the districts had protective GMTs for all serotypes (excepting serotype A for Tsirang) at the time of this study. It is generally accepted that a minimum of 80% of the population should be vaccinated in order to have an effective herd immunity to prevent disease transmission in a herd (Lombard and Shermbrucker, 1994). The vaccination coverage, in terms of protective immunity, was low, particularly in the endemic districts, since less than half of the vaccinated population had positive antibody titres. However, for Tsirang, the seropositivity, particularly for serotype A was high (88%). This differing seropositivity

could also be due to differences in the implementation of the actual vaccination programme in terms of the cold-chain maintenance and delivery mechanisms.

The immune response to Asia 1 serotype was poor in all districts. Serotypes A and Asia 1 have not been recorded in Bhutan since 2003 (Chapter 3). Therefore, given the disease's epidemiology, there is a need to reconsider the decision to use the trivalent vaccine, the cost of which is much higher than a monovalent vaccine.

The findings from this study are constrained by the fact that studies such as this can only provide a snap-shot of the immune status at one point in time. In order to thoroughly understand the complete kinetics of the immune response to FMD vaccine under field conditions in Bhutan, it is necessary to undertake a properly designed vaccine-efficacy study. For instance, a longitudinal study needs to be done with a FMD-naïve population wherein animals selected randomly should be identified and vaccinated and then sampled at regular intervals. There is also a need to undertake vaccine matching studies to ensure that the virus strains used in the vaccine are homologous to the ones circulating in the population. Owing to constraints with funds and time, such a study could not be carried out. However, this study has paved the way for further studies to provide a comprehensive understanding of the kinetics of immune response in the vaccinated cattle population of Bhutan.

In conclusion, the study showed differing immune responses between endemic and disease-free districts. Except for serotype A in Tsirang district, none of the vaccinated herds had protective “herd” immunity. It is recommended that a longitudinal vaccine efficacy trial be designed and implemented to understand the kinetics of the immune response under field conditions in Bhutan.

CHAPTER ELEVEN

General Discussion

Foot-and-mouth disease is a highly contagious viral disease that is endemic in many parts of the world, particularly in developing countries of Asia, Africa, the Middle East, and some parts of Europe. The economic impact of FMD is more apparent in countries where livestock exports constitute a significant part of the country's economy. On the contrary, the economic impact may not be easily quantifiable in countries that follow an extensive system of livestock farming or where the country's economy is not dependent upon livestock export earnings.

With 90% of the agrarian population in Bhutan dependent upon livestock (Anonymous, 2007a), particularly cattle, for their livelihood, diseases such as FMD can have a significant impact on the livelihood of farmers through loss of draught power, deaths (especially calves), reduced milk production, and restrictions on the sale of livestock and livestock products (Chapters 4 and 5). For instance, FMD caused an economic loss of approximately USD 2 million a year in the early 1990's in Bhutan (Tshering, 1995). Therefore prevention and control of FMD is in the general interest of the livestock farmers in particular, and the country at large. Effective control of FMD can only be achieved if it is based on a clear understanding of the epidemiology of the disease (Moutou, 2002, Perry *et al.*, 2002, Rweyemamu *et al.*, 2008a).

Prior to the research reported in this thesis, very limited or no information was available on the epidemiology of FMD in Bhutan with respect to its distribution, risk factors, the movement patterns of animals, and validity of the passive surveillance system. Although, the NFMDCP had been in operation since 1996, little progress was made in disease control

primarily due to a lack of epidemiological information. In the current study a series of retrospective and prospective studies were conducted to understand the disease's distribution, its persistence, risk factors, and surveillance aspects of FMD in Bhutan.

11.1 Distribution and persistence of FMDV

Although FMD is endemic in Bhutan, the sub-districts and districts bordering India appear to be at more risk of outbreaks than do the interior districts. The findings of the retrospective (Chapter 3) and the seroprevalence studies (Chapters 6 and 7) support the hypothesis that the sub-districts and districts bordering India act as primary endemic areas (areas for disease persistence). Although vaccination is undertaken bi-annually in these high risk areas, outbreaks continue to occur (Chapter 4). The reason for this could be multiple. It is likely that the single most important reason for these outbreaks is the unrestricted mixing of animals at the border areas across hundreds of unofficial entry points along the border (Chapters 4, 5 and 8). There is virtually no control over this practice and since vaccination doesn't provide sterilising immunity (Doel, 2003, Grubman and Baxt, 2004), vaccinated animals can acquire infection. Similar observations of higher incidences of outbreaks of FMD were also noted along the international borders of Brazil owing to uncontrolled animal movements and lack of surveillance activities (Mayen, 2003). The interior districts appear to act as secondary endemic areas (areas for disease propagation) where, owing to limited vaccination coverage (Chapters 4, 7, and 10) and unrestricted animal movements at all levels (Chapters 5, 7, and 8), the disease propagates. Cattle are the most susceptible species while small ruminants and pigs seem to play minor roles in the disease's epidemiology in Bhutan (Chapters 3, 5 and 6). However, small ruminants, in particular, can be infected through contact with cattle (Chapter 6). Therefore in the future unvaccinated small ruminants could be used as tracers (OIE, 2009a) as has

been reported elsewhere (Ranabijuli *et al.*, 2010). Given the extremely low numbers of pigs, their system of management, and their inability to remain as carriers, these animals do not seem important in the epidemiology of FMD in Bhutan as has been reported in northern Thailand where similar husbandry practices are adopted (Chamnanpood *et al.*, 1995). Similar observations of higher incidences of FMD in cattle, in comparison with other species, have also been reported in the neighbouring Indian states of Assam (Sarma and Sutopa, 2003) and West Bengal (Bhattacharya *et al.*, 2005). Although all four serotypes have been recorded in Bhutan, type O was the most prevalent and C the least prevalent serotype. A similar distribution of serotypes has been noted in West Bengal (Bhattacharya *et al.*, 2005) and Assam (Sarma and Sutopa, 2003). In the years 2000-2006 and 2003-2004 in the Indian states of Uttar Pradesh and Kashmir, respectively (Sharma and Kakker, 2005, Verma *et al.*, 2008) serotype O also was the most prevalent serotype followed by serotypes A and Asia 1. Serotype C was not recorded during this period (Sharma and Kakker, 2005, Verma *et al.*, 2008). Of the outbreaks occurring between 1987 and 1997 in Bangladesh, serotype O was also the most dominant serotype followed by A and Asia 1 (Islam *et al.*, 2001). In Nepal, serotype O is also the most dominant serotype whereas serotypes A, Asia 1 and C were last recorded in 1997, 1997 and 1996, respectively

(http://www.wrlfmd.org/fmd_genotyping/asia/nep.htm, accessed on 12 January 2011).

Waves of outbreaks of FMD occurred in Bhutan every four to five years, as a result of incursion of the PanAsia strain of the O serotype, possibly through the transboundary movement of animals (Chapters 3 and 4). Phylogenetic studies conducted by the WRLFMD showed that the viruses from Bhutan were closely related to the ones circulating in India and Nepal, the two countries from which Bhutan largely imports its cattle (Anonymous, 2011). Similar observations of the cyclical occurrence of FMD every

three to four years has been reported in Colombia in an endemic setting (Gallego *et al.*, 2007). This cyclic occurrence of outbreaks in epidemic proportions could be due to the waning of the immunity obtained from either natural infection or vaccination. Serum antibody levels can remain at protective titres for up to 4.5 years in convalescent cattle (Cunliffe, 1964, Salt, 1993) although vaccinal immunity lasts less than one year following a single vaccination (Salt, 1993). Although vaccination is routinely undertaken, its effectiveness is questionable given the numerous factors that could limit a successful vaccination (Garland, 1999, Doel, 2003). In Chapter 10 the low levels of protective immunity in vaccinated cattle, even when revaccinated every six months, was highlighted. Therefore, vaccinal immunity is expected to be quite low in the livestock population in Bhutan as was demonstrated in Chapters 4, 7 and 10.

The devastating effects of the PanAsia strain of the O serotype, even in an endemic setting, have been demonstrated for the first time in Bhutan (Chapter 4). Similar observations of high pathogenicity of the PanAsia strain of the FMDV have been reported in Lao PDR in 1999 (Perry *et al.*, 2002) and in the states of Punjab and Uttar Pradesh in India in 2008 (Singh *et al.*, 2008b). Khounsy *et al.* (2009) demonstrated that the ME-SA topotype PanAsia strain dominated type O outbreaks in Lao PDR from 1999 to 2004. Phylogeny studies showed that the PanAsia strain also dominated the type O outbreaks in India during the same period (Hemadri *et al.*, 2002, Knowles *et al.*, 2005). While weak immunity in the animals could have allowed the disease to spread rapidly within herds and villages, the poor biosecurity practices and poor nursing care of the sick animals increased the risk to susceptible animals. The level of biosecurity and movement control presently possible in Bhutan pointed to the need to aim for a high level of herd immunity (at least 80%) in cattle populations to reduce the spread and impact of FMD. In situations where surveillance and regulation of animal movements is difficult, as in Bhutan, vaccination will continue to be

an important tool to prevent and control FMD. This study also showed that the currently used 10 km radius for the control and surveillance zones may not be sufficient to prevent spread of infection during an outbreak of FMD. Such information needs to be included in the NFMDCP.

11.2 Livestock husbandry practices – the risk factors

The livestock husbandry practices currently adopted in Bhutan pose significant challenges to the control and prevention of FMD. In Chapters 5 and 7 the extensive system of livestock farming characterised by unrestricted movements and mixing of animals at all levels was highlighted. The practice of allowing animals to mix within and between herds and villages at grazing and watering areas, and the transhumance practice pose significant risks to the spread and persistence of FMD in Bhutan. Studies conducted in other FMD-endemic countries (Cleland *et al.*, 1996, Bronsvoort *et al.*, 2004a, Oo, 2010) also incriminate unrestricted movement and mixing of animals as one of the main reasons for the disease's endemicity in these areas. Practices such as feeding of kitchen wastes to cattle could also play a significant role in the disease's epidemiology in Bhutan. This warrants further investigation, especially in the wake of speculations of contaminated wheat straw as being responsible for the 2000 FMD epidemic in Miyazaki Prefecture in Japan (Sugiura *et al.*, 2001, Sakamoto and Yoshida, 2002). Risk-factor studies performed elsewhere have identified owning forest buffalo (*Syncerus caffer namus*) (Bronsvoort *et al.* 2004) or sheep and goats (Megersa *et al.* 2009), purchasing livestock at markets (Lindholm *et al.* 2007), the density of cattle herds (Perez *et al.* 2004), and close proximity to slaughter facilities (Lindholm *et al.* 2007), as risk factors that best explain the occurrence of FMD. These risk factors were not found to be important or relevant in the study presented here. This could

be due to differences in livestock husbandry practices, socio-economic conditions, people's dietary preferences, or geo-physical conditions of the study area and other areas.

The potential role of disease spread by the cattle herds migrating from one agro-ecological zone to another was demonstrated in Chapter 7. The significantly higher seroprevalence in the migratory herds both at the individual animal-level (24.8%, 95% CI: 20.6, 29.5) and herd-level (64.1%, 95% CI: 53.5, 73.9) as compared to the sedentary herds (17.5%, 95% CI: 15.6, 19.5 and 37.7%, 95% CI: 33.4, 42.2, respectively) underlines the importance of this livestock production system in the disease's epidemiology. The fact that pastoralists migrating to lower altitudes had higher seroprevalences reflects the endemicity of FMD in the sub-districts bordering India.

11.3 Animal movement patterns

Animal movements are considered one of the most important means for the spread of FMD, locally, regionally, and globally (Rweyemamu, 1984, Fèvre *et al.*, 2006). From the daily movements for grazing and watering purposes (Chapters 5 and 7) to the seasonal long-distance migration (Chapter 7) and the movements associated with livestock trading practices (Chapter 8), animal movements in Bhutan are found to occur in multiple forms and varying magnitude. In Chapter 8 the existence of complex animal movements spurred by livestock trading practices in two districts with differing disease statuses was illustrated. Farmers traded cattle mainly for the purpose of draught power for use in agriculture. However, the bulk of livestock trading, be it cattle, small ruminants or pigs, is spurred by the rising demand for meat by the burgeoning urban population. More trading occurred in the dry season (winter) due to increased demand for meat for festivals and religious ceremonies during the traditional New Year. Livestock trading is still unstructured and

much of it occurs without the knowledge and intervention of the regulatory authorities, especially the trading between villages and sub-districts. Notwithstanding the complex and non-transparent nature of animal movement, the study did show differing movement patterns between the FMD-endemic and FMD-free districts. For instance, there were more inward movements of all livestock species in the FMD-endemic district (Sarpang) as compared to the FMD-free district (Tsirang). The volume of animals traded was also much higher in Sarpang than in Tsirang district. The presence of numerous unofficial trading routes along the Indo-Bhutan border in Sarpang district could be an important determinant for the frequent incursion and persistence of FMD in this district. The findings from this study support the hypothesis that the disease's persistence in the southern districts could be as a result of unregulated mixing of animals along the porous border between India and Bhutan. Similar observations of complex animal movements in an FMD-endemic area as compared to an FMD-free area have also been demonstrated in Myanmar (Oo, 2010). In a study of the animal movement patterns in the Greater Mekong Sub-region (Cambodia, Lao PDR and Vietnam), Cocks *et al.* (2009b) also reported the dominance of unofficial livestock movements across international borders which posed significant risks to disease incursion in the importing countries. The greatest demand for livestock in these countries was also in the dry season coinciding with their traditional new year and local festivals (Cocks *et al.*, 2009b). There are, however, differences in the livestock trading practices and patterns between Bhutan and some countries in South-East Asia. For instance, Bhutan doesn't have livestock markets and there is virtually no export of live animals to any country. In SE Asia livestock markets play a critical role in the disease's epidemiology through the process of gathering, mixing and redistribution of livestock (Cocks *et al.*, 2009a). Livestock collection depots, especially in Cambodia, are also thought to play an important role in the disease's epidemiology, given the poor biosecurity practices followed

in these establishments (Cocks *et al.*, 2009b). Animal movements will continue to be a major stumbling block for successful disease control in Bhutan for many years to come and this study highlights the need to undertake further longitudinal studies to have a complete understanding of the livestock trading and movement patterns for the whole country.

11.4 Disease Surveillance and zoning prospects

In this thesis the use of clinical (farmer-diagnosed) and serological surveillance as important tools for routine surveillance of FMD in Bhutan has been outlined. Clinical observations of animals in the field can be an important source of surveillance data and such information gathered from the same country at different times can provide cumulative evidence of animal health status in that country (OIE, 2009b). The country's passive surveillance system, based on the farmer-diagnosed and reported incidences of FMD for the 13-year period, provided valuable information about the spatiotemporal distribution and other epidemiological features of the disease. A good network of veterinary centres covering even the remote parts of the country and manned by trained veterinarians ensured that outbreaks of FMD are reported regularly. There was, and still is, full support from the government for the control of FMD. Unlike many other neighbouring countries, the veterinary services, including vaccination, treatment, and even vaccines and medicines are provided free and therefore this is likely to act as an incentive to the livestock owners to report any outbreaks to the nearest veterinary centre. Analysis of passive surveillance data in other countries have yielded valuable information in understanding the disease's epidemiology (Bhattacharya *et al.*, 2005, Khounsy *et al.*, 2008, Verma *et al.*, 2008, Ayebazibwe *et al.*, 2010). Because of their close association with their animals, and since the clinical signs are easily discernible, especially in cattle (Davies, 2002), livestock

owners have good knowledge about the clinical and epidemiological features of FMD (Bronsvoort *et al.*, 2003, Rufael *et al.*, 2008, Oo, 2010).

Prior to this study, no active surveillance for FMD was conducted in Bhutan. The serological (Chapter 6) and questionnaire-based surveys (Chapters 5 and 7) support the findings of the passive surveillance system (Chapter 3) that districts and sub-districts that border India have a higher prevalence than the interior districts. Active serological and questionnaire-based surveys have also validated the usefulness of the country's passive surveillance system, particularly on the disease-free status of Tsirang district. In an epidemiological study to demonstrate freedom from disease in the Tanintharyi division in the Myanmar-Thailand-Malaysia zone, Oo (2010) also used active serological surveys complemented with the livestock owners' knowledge to validate the findings of the passive surveillance system. The findings of the current study have increased the level of confidence in the passive surveillance system currently adopted in Bhutan. However, the findings from passive surveillance should be backed up and complemented by active serological surveys, especially where the status of FMD-suspect cases needs to be clarified (OIE, 2009a). The active serological and questionnaire-based surveillance did show a higher sensitivity than the passive surveillance system by detecting active infections in herds and villages that, according to the official records, were considered not to have had an outbreak of FMD (Chapters 5 and 6).

Vaccination coverage, as determined by seropositivity to O serotype, was found to be very low in the migratory herds (21.1%) as compared with the sedentary herds (62.7%). However, even for the sedentary herds, except for antibodies to serotype A in Tsirang district (mean log titre=2.09), all other serotypes had titres below log 2.0 (1:100 titre) which is usually considered to be the protective titre in vaccinated cattle. Therefore, a relatively high proportion of cattle would be susceptible to infection should FMDV gain

entry into these populations as none of the districts had protective titres for all serotypes (excepting serotype A for Tsirang) at the time of this study.

As FMD is endemic in most districts in Bhutan, controlling the disease in the whole country would be very expensive and unrealistic, as has been shown over the years. This is further compounded by the fact that the country shares a long and porous border with the Indian states of Assam, West Bengal and Arunachal Pradesh where FMD is endemic (Sarma and Sutopa, 2003, Bhattacharya *et al.*, 2005, Sanjoy and Sarma, 2005). Bhutan imports most of its cattle, pigs, sheep and goats from India and therefore the risk of FMDV incursion is high. The imported animals are rarely subjected to stringent disease screening tests against FMD at the quarantine stations. There is also no follow-up on the movements of these imported animals once they enter the country (Chapter 8) due to the lack of animal identification and tracking systems. The practice of livestock migration, which is still common in most districts, places a significant challenge to the success of the present control methods (Chapter 7). Therefore, alternative approaches that are financially sustainable and technically sound are needed based on the disease's epidemiology. There is an increasing call for the use of progressive control of FMD using the concepts of zoning (Fujita, 2004, Rweyemamu *et al.*, 2008a). Zoning is an approach aimed at identifying geographical areas of varying disease status within a country or region for the purpose of trade, disease control and eradication (OIE, 2010). Therefore, there was a need to undertake studies to validate the disease-free status of Tsirang district which has been free from clinical FMD since 1998 (Chapter 3). Clinical and serological surveillance studies validated the FMD-free status of this district and have now paved the way for the initiation of progressive control of FMD through zoning. The whole of Tsirang district can now be designated as an eradication zone and the bordering districts (Sarpang,

Wangdue Phodrang, and Dagana) should be designated as a buffer zone. Separate control measures need to be adopted for these two different zones.

There is an increasing acceptance of the fact that progressive control of TADs, such as FMD, should be targeted at the source (endemic countries/regions) to achieve global FMD-risk reduction (FAO-OIE, 2004, Kitching *et al.*, 2007, Rweyemamu *et al.*, 2008b, Sumption *et al.*, 2008). Progressive control of FMD should be based on sound epidemiological assessment of the incidence and distribution of FMD in a country or a region (Rweyemamu *et al.*, 2008b). Given the lack of epidemiological data, especially on spatiotemporal distribution of FMD in South Asia (Rweyemamu *et al.*, 2008b) and SE Asia (Gleeson, 2002), the findings from this study are expected to add to the repository of knowledge on the epidemiology of FMD in South Asia and the rest of the world thereby contributing to the initiation of control approaches based on the disease's epidemiology. This study has, for the first time, produced information about the distribution, risk factors, seroprevalence, animal movement patterns, and validation of passive surveillance for FMD in Bhutan. The success in the control of FMD in South America was based on the premise that control schemes had to be based on eco-systems that took into account the dynamics of FMD, the farming systems and cattle movements to identify primary and secondary endemic areas (Rweyemamu and Astudillo, 2002). The current study has unravelled the epidemiology of FMD in Bhutan and has paved the way for initiation of control approaches based on the epidemiology of the disease and the farming system adopted in Bhutan.

11.5 Limitations of this study

This study was not without its limitations and constraints. Firstly, the retrospective studies (Chapter 3) were based on the official records of outbreaks of FMD. Not all outbreaks that

occurred in the field would have been reported and consequently there is the potential for underreporting of cases as is inherent in a passive surveillance system (McLeod, 2003, Thrusfield, 2005, Oo, 2010). However, given the fact that FMD produces characteristic clinical signs and the good coverage of veterinary services, even in the remote villages, underreporting may not be common. As treatment and vaccination is provided free by the government, farmers usually report outbreaks of diseases in their herds and villages to attract timely attention from the authorities.

The methodology for the prospective studies was a combination of questionnaire interviews and NSP serology. As with all questionnaire-based studies, some of the limitations could include the influence of recall bias, interviewer bias and failure to validate the questionnaire responses by repeating the questionnaire survey among the same respondents (Bronsvort *et al.*, 2003). Recall bias is less likely to occur as FMD produces characteristic clinical signs, especially in cattle (Davies, 2002). Cattle constituted the majority of the FMD-susceptible species in the study area and therefore the disease could be easily recognised (diagnosed) by the farmers and field veterinarians. This was confirmed by the finding that the majority (93%) of the respondents could recognise the clinical signs or lesions of FMD when a picture of FMD was shown. Similarly, 87% of the respondents could recall some of the clinical signs and lesions of FMD. The questionnaires were also pre-tested with several farmers before a final version was produced to minimise misunderstanding of the questions.

None of the currently available NSP ELISAs have a 100% sensitivity and specificity. Thus, misclassification of animals, herds, or villages is likely if based on NSP serology alone. However, at least for the disease freedom study, false positive results would be highly unlikely as three ELISAs were used in series. Diagnostic tests when used in series improve the test specificities and therefore reduce the number of false positives (Paton *et*

al., 2006). The combined use of clinical and serological surveillance, especially for the disease freedom study, helped determine that the serological results were most likely false positives.

Sampling from the migratory herds was conducted opportunistically when herds were available and therefore selection biases could have occurred. Given the highly mobile nature of the pastoralists and the scattered location of the villages, it was not possible to interview and sample as many farmers and animals, respectively, as was initially envisaged.

The scope of the immune status study was limited due to financial, logistical, and time constraints and therefore only a cross sectional study could be undertaken. A longitudinal study of at least one year would have produced better information on the change in the immune response in the vaccinated population in the field.

Another limiting factor for this study was the lack of clear delineation of the village boundaries. There were differences in the number and names of villages in each district based on records obtained from different government sources. None of these were in complete agreement to what was defined as a village on the ground. Some of the villages listed in the official lists were missing (when counterchecked physically).

11.6 Recommendations

Based on the findings of this study, the following recommendations are proposed to further strengthen the current NFMDCP in Bhutan.

- **Active surveillance**

The sub-districts and districts adjoining India are at significantly higher risk of FMDV infection than are the interior districts. Therefore, disease surveillance activities, including active serological and clinical surveillance, need to be

strengthened in these areas to facilitate early detection and imposition of control measures. Disease control activities need to be strengthened in these primary endemic areas (areas of disease maintenance) so that the risk for further spread into the interior parts of the country can be reduced. Arrangements have to be made with BAFRA so that all FMD-susceptible animals imported into the country are screened with an NSP ELISA before the animals are released.

- **Risk-based disease control**

All sub-districts adjoining India need to be classified as a 'Buffer zone' and intensive vaccination and movement control should be undertaken in these sub-districts. Bi-annual vaccination should be compulsory in these sub-districts to thwart any incursion of FMDV across the border. Cattle are the priority species for disease control in Bhutan given their large population and species susceptibility. Small ruminants and pigs, because of their relatively small numbers and the husbandry/management practices adopted by farmers for these species, do not seem to play a significant role in the disease's epidemiology. Therefore, control programmes should be focussed on cattle.

There is a need to develop customised control programmes for the migratory herds given their mobile nature and the higher risk associated with this practice. For instance, greater awareness needs to be created amongst the pastoralists about the basic biosecurity measures to be adopted while on migration. Compulsory vaccination, at least one month before migration, would significantly reduce the risk of FMDV infection on migration.

- **General awareness programme**

This study has shown that farmers that reside in villages/districts that had no outbreaks of FMD for many years were less likely to identify and diagnose FMD than those residing in FMD-endemic districts. For instance, many farmers in the FMD-affected villages of Zhemgang in 2007 (Chapter 4) had never previously seen FMD and therefore misdiagnosed the disease and were late in reporting it to the veterinary authorities. Farmers in Tsirang district (Chapter 9) were also less likely to diagnose FMD. Therefore, there is a need to create awareness about FMD in villages/areas where FMD had not occurred for many years.

- **Livestock identification and tracking system**

The animal movement study (Chapter 8) highlighted the widespread movement of animals within villages, between villages, between sub-districts and between districts. Pastoralism was found to still be thriving in the study districts with tens of hundreds of cattle moving between the sub-tropical and temperate zones every year. Unless, these movements are identified and monitored, no amount of other disease control measures will be able to prevent the occurrence of outbreaks. It is therefore crucial to implement an animal identification system, at least for cattle, to track the movements of all cattle within the country.

- **Progressive control approaches using zoning**

As FMD is endemic in most districts, and given the unrestricted movement of animals at all levels, the present control strategies may not be effective in reducing the incidence of disease outbreaks. The ‘blanket’ vaccination approach used at the

moment is too expensive and is not effective. Therefore, new approaches, using the concept of progressive zoning, need to be initiated. Tsirang district is free from active FMDV infection and, given the geographical features, there is a good potential to maintain this status. For the moment, the whole of Tsirang district can be declared as an eradication zone and the bordering districts declared buffer zones. Animal movements into Tsirang should be monitored closely and active surveillance should be conducted at all entry routes into Tsirang. Intensive vaccination at the border areas between Tsirang and the three endemic districts of Sarpang, Wangdue Phodrang and Dagana should be implemented so that there is progressive control of FMD. The disease free zone should be slowly expanded in the future depending on the success of the control programme.

Similar approaches should be initiated in districts/sub-districts that have had no outbreaks of FMD for many years, after necessary evaluation and validation of their disease-free status.

11.7 Further Research

In order to understand the complete epidemiology of FMD in Bhutan, further research needs to be done in several areas.

- **Molecular epidemiological studies**

Due to the high mutation rate, the FMDV is known to have a high level of antigenic variation. Virus genome sequencing and antigenic variation studies have been invaluable in understanding the molecular epidemiology of the disease in determining the source of outbreaks. Therefore, molecular studies (phylogeny) need to be undertaken for all outbreaks occurring in the country to investigate the

source of outbreaks and assess the antigenic variation of the FMDV from different areas in the country and at different times.

- **Vaccine efficacy studies**

The cross sectional immune study showed low levels of immune response in the vaccinated population, especially in the FMD-endemic areas. There is a need to undertake longitudinal studies to determine the kinetics of the immune response to vaccination under field conditions in Bhutan. All factors that are determinants of effective vaccination, including the vaccine, cold chain maintenance, and vaccine delivery mechanisms, should be studied to have a comprehensive understanding of the vaccination programme in Bhutan.

Vaccine matching tests should be done for all vaccines to ensure that the FMDV antigens used in the commercial vaccines are closely related to the field strains of virus circulating in Bhutan.

- **Risk analysis**

As Bhutan imports large amounts of raw beef and pork from India and other neighbouring countries, the risk associated with this has not been previously investigated. Therefore a risk analysis needs to be undertaken to quantify the risks associated with the importation of unprocessed beef and pork into the country.

- **Role of wildlife in disease epidemiology**

With more than 70% of the country under forest and 26% of the total area reserved as biological corridors, there are numerous cloven-hoofed wild animals in Bhutan

such as barking deer (*Muntiacus muntjak*), spotted deer (*Axis axis*), sambar deer (*Rusa unicolor*), wild pigs (*Sus scrofa*), wild cattle (*Bos gaurus*), takin (*Budorcas taxicolor*), and blue sheep (*Pseudois nayaur*). As most of the villages adjoin the forests, instances of human-wildlife conflict/interaction is very common (Wang and Macdonald, 2006, Sangay and Vernes, 2008). Therefore, it is not uncommon for domestic and wild animals to share grazing grounds or to mix at some point in time. Anecdotal evidence (Chapter 4) suggests that wildlife are a source of FMD for the domestic animals. Therefore, there is a need to clarify the role played by wild animals in the epidemiology of FMD in Bhutan.

11.8 Conclusions

In conclusion, the epidemiology of FMD in Bhutan mirrors the disease epidemiology in the South Asian region. Foot-and-mouth disease was found to be endemic and periodically epidemic in Bhutan. The endemicity of the disease, coupled with limited resources, warrants a change in the approaches for the control of FMD in Bhutan. In line with the global and regional approaches of progressive control of FMD, it is recommended to initiate a zoning approach as an alternative means for controlling FMD in Bhutan. The control of FMD in Bhutan will have far-reaching benefits to the country at large and the livestock farmers, in particular, by enhancing livestock production and household income.

List of Appendices

Appendix 1

Questionnaire for herd-level risk factor study of Foot-and-Mouth Disease in Bhutan

District: Geog: Village:

Altitude (metres above sea level) Date:

Name of Interviewer:

Can the farmer diagnose the disease? Yes [] No []

1. Background profile

1.1 Name of the respondent. 1.2 Gender: M F

1.3 No of household members 1.4 Educated: Yes [] No []

1.5 Total Land: Acres

1.6 Main source of income: (*Rank in order of importance from 1 to 3; 1 being the most important*)

a) Agricultural farming []

b) Livestock farming []

c) Paid Casual worker []

d) Others (*please specify*)

.....

2. Livestock population profile (Write the total figures in the box)

2.1 Cattle	<input type="text"/>	2.2 Buffalo	<input type="text"/>	2.3 Sheep	<input type="text"/>
2.4 Goats	<input type="text"/>	2.5 Pigs	<input type="text"/>	2.6 Horses	<input type="text"/>
2.7 Poultry	<input type="text"/>				

3. Livestock husbandry system

3.1 How long have you been undertaking livestock farming? Years

3.2 How long have you been living in this village? Years

3.3 Why do you keep cattle? (*Rank in order of importance from 1 to 4; 1 being the most important*)

(a) Dairy production []

(b) Draught power for ploughing field []

(c) For manuring the field []

(d) For sale during urgent need of cash []

(e) Other (*please specify*)

.....

3.4 Housing

a) How are the animals housed during the day and night time? (*Please use the codes below*)

CODES

I. Open air tethering near the house

II. Free ranging near the house

III. Always housed in a shed or a pen

(i) Livestock housing during day (*please tick all that apply*)

Livestock species	I	II	III
Cattle / buffalo			
Sheep and goats			
Pigs			

(ii) Livestock housing at night (*please tick all that apply*)

Livestock species	I	II	III
Cattle / buffalo			
Sheep and goats			
Pigs			

3.5 Feeding

a) What do you feed your cattle / buffalo? (*please tick all that apply*)

- (i) Commercial feed [] (iv) Grazing near the house []
(ii) Kitchen waste [] (v) Grazing in the forest []
(iii) Crop by-products [] (vi) Other (please specify).....

b) What do you feed your sheep and goats? (*please tick all that apply*)

- (i) Commercial feed [] (iv) Grazing near the house []
(ii) Kitchen waste [] (v) Grazing in the forest []
(iii) Crop by-products [] (vi) Other (please specify)

c) What do you feed your pigs? (*please tick all that apply*)

- (i) Commercial feed [] (iv) Free ranging near the house []
(ii) Kitchen waste [] (v) Free ranging in the village []
(iii) Crop by-products [] (vi) Other (please specify)

3.6 Source of drinking water

a) What is the source of drinking water for cattle? (*Please tick all that apply*)

- (i) Tap water [] (iv) Near by spring water []
(ii) River / Stream []
(iii) Irrigation channels [] (v) Other (*please specify*)

b) Do your cattle mix with cattle owned by other farmers from your village at the water source?

Yes [] No []

If No, go to section 3.7

If Yes,

(i) With how many herds do they mix at the water source? (Please tick one)

1 to 5 herds [] 6 – 10 herds [] > 10 herds []

3.7 Management of animal wastes

a) What do you do with the cattle dung? (*Please tick all that apply*)

(i) Leave in the shed (iii) Transfer to field for use as manure

(ii) Place in a disposal pit (iv) Sell it

(b) Do you use cattle dung from your neighbour for agricultural purposes?

Yes No

4. Disease epidemiology

4.1 Has there been any outbreak of foot-and-mouth disease in Your Village within the last 5 years?

Yes No Can't remember

If No or Can't remember, Go to section 4.5

4.2 If Yes, When was the last FMD outbreak in your village? (*please tick one*)

a) Less than a year ago

b) More than 1 year ago

c) More than 2 years ago

d) More than 3 years ago

e) Can't remember

4.3 How many cattle were affected during that outbreak in your village? (*please tick one*)

a) All the cattle in the village

b) Half of the cattle population

c) A few animals

d) Can't remember

4.4 What do you think was the source of the disease? (*please tick all that apply*)

a) Import of meat from infected village (s)

b) Import of cheese and butter from infected village

c) Movement of animals

d) Don't know

e) Other (*please specify*)

4.5 Have you ever had an outbreak of FMD in Your Herd within the last 5 years?

Yes []

No []

Can't remember []

If No or Can't remember, go to section 5

If Yes,

a) When was the last outbreak in your herd? (*please tick one*)

i) Less than a year ago []

ii) More than 1 year ago []

iii) More than 2 years ago []

iv) More than 3 years ago []

b) How many of your animals were affected in total in that outbreak?

i) Cattle

ii) Buffalo

iii) Pigs

iv) Sheep and goats

c) Did any of your animals die in that outbreak?

Yes []

No []

If Yes,

How many of your animals died in that outbreak?

i) Cattle

ii) Pigs

iii) Sheep and goat

d) Which one of the following actions did you take once your animals got FMD? (*please ask question and tick all that apply*)

i) Informed the village headman []

ii) Informed the local Veterinary centre for treatment []

iii) Used traditional medicine to treat []

iv) Left the animals to heal by themselves []

v) Allowed the animals to graze freely in the village []

vi) Sold the animal []

e) What are the difficulties you face when your animals are affected with FMD? (*please tick all that apply*)

- (i) Loss of income due to reduced milk production []
- (ii) Cannot use bullocks for ploughing []
- (iii) Loss of calves []
- (iv) Waste of time to look after sick animals []
- (v) Other (*please specify*)

f) How often do you usually see FMD in your herd?

- (i) Twice a year []
- (ii) Once in a year []
- (iii) Once every 2-3 years []
- (iv) Once every 4-5 years []
- (v) Once every 6-10 years []
- (vi) Can't remember []

h) In which season/month is FMD found to occur most commonly? (*note either the month or the season*)

.....

5. Vaccination Status

5.1 Are your cattle vaccinated against FMD?

Yes [] No [] Not Sure []

If No, state the reasons for not vaccinating?

.....

If Yes,

5.2 How often are your animals usually vaccinated against FMD?

- a) Twice a year []
- b) Once a year []
- c) Once every 2-3 years []
- d) Can't remember []

5.3 Do you believe the FMD vaccine protects your animals against FMD?

Yes [] No [] Not Sure []

If No or Not Sure go to 5.4

a) If Yes, in what way do you think it protect your animals?

(i) My animals do not get the disease at all even when there is disease in the village []

(ii) Even if infected, animals only develop mild lesions and recover quickly []

(iii) Other (*please specify*)

.....

5.4 During each vaccination programme, how many of your animals are vaccinated?

a) Few only []

b) Half of the herd []

c) Entire herd []

d) Can't remember []

6. Livestock and Livestock products movements

6.1 Are your animals sent for grazing? Yes [] No []

If No go to section 6.2

If Yes,

a) What species of animals are sent for grazing? (*please tick all that apply*)

(i) Cattle []

(ii) Buffalo []

(iii) Sheep and goats []

b) Where do you send your animals for grazing? (*please tick all that apply*)

(i) To the forest area []

(ii) To the community grazing ground (*Tsamdro*) []

(iii) Along the border area (applicable for villages near Indian border only) []

(iv) Other (*please specify*)

b) Why do you send your animals for grazing? (*please tick all that apply*)

(i) Shortage of feed or crop by products []

(ii) Cannot afford to buy feed []

(iii) Herd size too large to manage on stall feeding alone []

(iv) Others (*please specify*)

c) In a year, how many months are the animals sent for grazing?

..... Months

d) On an average, with how many herds from the same village do they mix during grazing in a day?

i) None []

ii) 1 to 5 herds []

iii) 6 to 10 herds []

iv) > 10 herds []

e) Do your animals mix with herds from near-by villages?

Yes [] No [] Not Sure []

If Yes, with how many near-by villages do your animals share grazing?

i) 1 to 5 villages []

ii) 6 to 10 villages []

iii) > 10 villages []

f) Do your animals mix with animals from India at the grazing ground?

Yes [] No [] Not Sure []

(*this is applicable only for villages bordering with India*)

g) Do your cattle graze together with sheep and goats?

Yes [] No [] Don't know []

6.2) If your bullocks become sick, do you hire bullocks from your neighbour for urgent agricultural works?

Yes [] No []

6.3) Do you give your bullocks or hire them to your neighbours or other farmers when their animals are sick?

Yes []

No []

6.4) Where do you usually buy your animals from? (*Please tick all that apply*)

a) From the same village []

b) From another village in the same district []

c) From another district []

d) From India []

e) Don't buy at all []

6.5) Do you sell your animals?

Yes []

No []

If No, go to section 6.6

If Yes,

a) Where do you normally sell your animals? (*Please tick all that apply*)

i) The same village []

ii) Different village in the same district []

iii) Different district []

iii) Other (*please specify*)

b) When do you sell your animals? (*Please tick all that apply*)

i) When in need of cash []

ii) When animals are diseased []

iii) When there are too many to manage []

iv) When they are too old and unproductive []

v) Others

.....

6.6) What kind of livestock products do you sell? (*Please tick all that apply*)

- a) Cheese / butter []
- b) Milk []
- c) Wool []
- d) Meat []
- e) None sold []

If none sold, go to sub-section 6.8

6.7) Where do you sell your livestock products?

- a) From my house []
- b) In the local market []
- c) To the middle man who then takes to market []

6.8) Where do you get your fresh meat and dairy products for home consumption from?

6.8.1 During normal

time:.....

6.8.2 During Losar/Puja or other important occasions

.....

6.9 Do you go on migration (Transhumance)?

Yes [] No []

Knowledge on Prevention and Control of FMD
--

7.1 What are the clinical signs of FMD?

.....

7.2 What do you think is the best way to prevent the spread of FMD in your village?

.....

7.3 Can you name any wild animals that also get FMD?

.....

7.4 Where do you usually get information on livestock diseases from?

- a) Neighbours []
- b) The livestock extension centre (LEC / RNR Centre) []
- c) Radio []
- d) Newspapers []
- e) Other (please specify)

.....

General information

8.1 Where is the nearest Livestock extension centre (LEC) located?

Village Geog

8.2 How long does it take to reach the nearest LEC? *(please fill in and tick the appropriate time description)*

a) By walking Minutes/Hours/Days

b) By vehicle Minutes/Hours/Days

8.3 How long does it take to reach the nearest motorable road from your house?

..... Minutes/Hours/Days

Appendix 2

Questionnaire survey for risk factor analysis of Foot-and-Mouth Disease in the migratory herds of Bhutan

District: Geog: Village:

Altitude (metres above sea level) Date:

Name of Interviewer:

Can the farmer diagnose the disease? Yes [] No []

1. Background profile

1.1 Name of respondent..... 1.2 Gender M F

1.3 No of household members 1.4 Educated Yes [] No []

1.5 Total Land:..... Acres

1.6 Main source of income: (*Rank in order of importance from 1 to 3; 1 being the most important and 3 being the least important*)

a) Agricultural farming []

b) Livestock farming []

c) Other (*please specify*).....

2. Livestock population profile

2.1 Cattle	<input type="text"/>	2.2 Yaks	<input type="text"/>	2.3 Goats	<input type="text"/>
2.4 Poultry	<input type="text"/>	2.5 Pigs	<input type="text"/>	2.6 Horses	<input type="text"/>
2.7 Sheep	<input type="text"/>				

3. Livestock husbandry system

3.1 How long have you been undertaking livestock farming? Years

3.2 How long have you been living in this village?Years

3.3 Why do you keep livestock? (Rank in order of importance from 1 to 4; 1 being the most important and 3 being the least important)

- (a) Dairy production []
- (b) Draught power for ploughing field []
- (c) For manuring the field []
- (d) For sale during urgent need of cash []
- (e) Others (please specify)

.....

3.4 Housing

a) How are the animals housed during the day and night time? (Please use the codes below)

CODES

- I. Open air tethering near the house
- II. Free ranging near the house
- III. Always housed in a shed or a pen

Livestock housing during day (*please tick all that apply*)

Livestock species	I	II	III
Cattle/yaks			
Sheep and goats			
Pigs			

Livestock housing at night (*Please tick all that apply*)

Livestock species	I	II	III
Cattle/yaks			
Sheep and goats			
Pigs			

3.5 Feeding

a) What do you feed your animals? (*please tick all that apply*)

- | | | | |
|------------------------|-----|------------------------------------|-----|
| (i) Commercial feed | [] | (iv) Free ranging near the house | [] |
| (ii) Kitchen waste | [] | (v) Grazing in the forest only | [] |
| (iii) Crop by-products | [] | (vi) Others (please specify) | |

3.6 Source of drinking water

a) What is the source of drinking water for the animals? (*Please tick all that apply*)

- | | | | |
|---------------------------|-----|-------------------------------------|-----|
| (i) Tap water | [] | (iv) Near by spring water | [] |
| (ii) River /Stream | [] | (v) Other (<i>Please specify</i>) | |
| (iii) Irrigation channels | [] | | |

b) With how many herds from the same village do your animals mix at the water source?

- None [] 1 to 5 herds [] 6 – 10 herds [] > 10 herds []

4. Disease epidemiology

4.1 Has there been any outbreak of foot-and-mouth disease in Your Village within the last 5 years?

[] Yes [] No Can't remember []

If No or Can't remember, Go to section 4.3

4.2 If Yes, When was the last FMD outbreak in your village? (please tick one)

- i) Less than a year ago []
- ii) More than 1 year ago []
- iii) More than 2 years ago []
- iv) More than 3 years ago []
- v) More than 4 years ago []
- vi) Can't remember []

4.3 Has there been any case of foot-and-mouth disease in Your Herd within the last 5 years?

[] Yes [] No Can't remember []

If No or Can't remember, Go to section 5

If Yes,

a) When was the last FMD outbreak in your herd?

- (i) Less than a year ago []
- (ii) More than 1 year ago []
- (iii) More than 2 years ago []
- (iv) More than 3 years ago []
- (v) More than 4 years ago []
- (vi) Can't remember []

b) How many animals were affected in total in the most recent outbreak that occurred in your herd?

Cattle ii) Yaks iii) Pigs

Sheep and goats

g) How often do you usually see FMD in your herd?

- (i) Twice a year []
- (ii) Once in a year []
- (iii) Once every 2-3 years []
- (iv) Once every 4-5 years []
- (v) Once every 6-10 years []
- (vi) Can't remember []

h) In which season/month is FMD found to occur most commonly? (*note either the month or the season*)

.....

5. Vaccination Status

5.1 Are your animals vaccinated against FMD?

Yes [] No [] Not Sure []

If No, state the reasons for not vaccinating?

.....

If Yes,

5.2 How often are your animals usually vaccinated against FMD?

- a) Twice a year []
- b) Once a year []
- c) Once every 2-3 years []
- d) Can't remember []

5.3 Do you believe the FMD vaccine protects your animals against FMD?

Yes [] No [] Not Sure []

If No or Not Sure, go to section 6

a) If Yes, in what way do you think it protect your animals?

- (i) My animals do not get the disease at all even when there is disease in the village []
- (ii) Even if infected, vaccinated animals develop mild lesions and recover quickly []

(iii) No outbreak after vaccination []

(iii) Other (*please specify*)

.....

6. Livestock and Livestock products movements

6.1 Are your animals sent for grazing? Yes [] No []

If No go to section 7

If Yes,

a) Where do you send your animals for grazing? (*tick all that apply*)

(i) To the forest area []

(ii) To the community grazing ground (Tsamdro) []

(iii) Other (*please specify*)

b) Why do you send your animals for grazing? (*tick all that apply*)

(i) Shortage of feed or crop by products []

(ii) Cannot afford to buy feed []

(iii) Cannot manage the animals because of large herd size []

(iv) Other (*please specify*).....

c) On an average, with how many herds from the same village do they mix during grazing in a day? (*select one*)

(i) None [] ii) 1 to 5 herds [] iii) 6 to 10 herds []

(iv) > 10 herds []

d) Do your animals mix with herds from near-by villages?

Yes [] No [] Not Sure []

If No or Not Sure, go to sub-section (e)

If Yes, with how many near-by villages do your animals share grazing?

(i) 1 to 5 villages []

(ii) 6 to 10 villages []

(iii) > 10 villages []

e) Do your cattle graze together with sheep and goats?

Yes [] No [] Don't know []

7. MIGRATION

7.1 Where do you migrate to (Final destination of migration)?

Village: Geog: District:

7.2 How long does it take to reach the final destination?

..... Days/Months (Please tick the appropriate unit)

7.3 Which month of the year do you leave for migration? (Tick one)

Jan [] Feb [] Mar [] Apr [] May [] Jun []
Jul [] Aug [] Sep [] Oct [] Nov [] Dec []

7.4 Why do you migrate? (Please rank in order of importance from 1 to 4; 1 being the most important and 4 being the least important)

- (i) Shortage of fodder []
- (ii) To avoid cold weather []
- (iii) For better economic opportunities []
- (iv) Traditional practice []
- (v) Other reasons.....

7.5 How do you migrate? (Tick all that apply)

- (i) By walking the entire route
- (ii) By transporting the animals in a truck
- (iii) By using both

7.6 What month of the year do you reach back to your village? (Tick one)

- Jan [] Feb [] Mar [] Apr [] May [] Jun []
Jul [] Aug [] Sep [] Oct [] Nov [] Dec []

7.7 Do you take all the animals in the herd for migration? Yes [] No []

If No,

Which categories of cattle are taken for migration?

- a) Milk [] b) Dry [] c) Pregnant [] d) Heifers []
- e) Calves [] f) Bulls []

7.8 With how many other households in the village do you migrate together?

- (i) None [] (ii) 1 – 5 households [] (iii) 6 – 10 households []
- (iv) > 10 households []

7.9 Please list the main geogs that your herd passes through while on migration until you reach your final destination.

.....

7.10 Do you follow the same route when you return back?

Yes []

No []

If No, List the main geogs that your herd passes through while returning back to your village

.....

7.11 During migration, do you come across cattle from other villages along the way?

Yes []

No []

7.12 During migration, which of the following wild animals have you come across? (*Tick all that apply*)

(i) Deer [] (ii) Wild pigs [] (iii) Wild cattle [] (iv) Others

(v) None []

7.13 During migration, what are the common diseases that affect your animals?

.....

7.14 In the most recent migration, did any cattle die along the way?

If No, go to next sub-section

If Yes,

What were the age groups of the dead animal? (*please tick all that apply*)

Adults [] Calves [] Can't remember []

What do you think was the cause of the death?

a) Predation by wild animals [] b) Disease [] c) Accidents []

What did you do with the carcass?

a) Ate it [] b) Sold it [] c) Buried []

d) Just left it in the forest [] e) Can't remember []

7.15 In the last 10 years, has the number of animals, in your herd, that died during migration increased or decreased?

Increased [] Decreased [] Stayed the same []
Don't Know []

If decreased, what could be the possible reasons?

a) Decrease in the wildlife predators [] b) Better health care facilities []

c) Others:.....

7.16 Have you ever passed through a village or geog affected with FMD during your migration during the last 5 years?

Yes [] No []

If Yes, Please name the village/geog

.....

7.17 Did your animals ever suffer from FMD during migration within the last 5 years?

Yes [] No []

If Yes,

What did you do? (*Tick one*)

- (i) Continued with migration until I reached the destination
- (ii) Waited until all animals recovered from the disease
- (iii) Others.....

7.18 Are your animals usually vaccinated against FMD before migration?

Yes [] No [] Can't remember []

If Yes,

(i) How long before migration do you vaccinate the animals?

..... Days/Months before the actual day of migration

If No

(i) Are there any specific reasons for not vaccinating your animals against FMD prior to migration?

.....

7.19 Do you vaccinate your animals before they return back to your village?

Yes No

7.20 How long do you stay away from your village while on migration

..... Months

7.21 Do you inform the nearest livestock office before you migrate? Yes [] No []

9. Knowledge on Prevention and Control of FMD

9.1 What are the clinical signs of FMD?

.....

9.2 What do you think is the best way to prevent the spread of FMD in the village?

.....

9.3 Can you name any wild animals that also get FMD?

.....

9.4 Where do you usually get information on livestock diseases from?

- a) Neighbours []
- b) The livestock extension centre (LEC / RNR Centre) []
- c) Radio []
- d) Newspapers []
- e) Other (please specify) []

.....

10. General information

10.1 Where is the nearest Livestock extension centre (LEC) located?

Village Geog

10.2 How long does it take to reach the nearest LEC?

a) By walking Minutes/Hours/Days

b) By vehicle Minutes/Hours/Days

10.3 How long does it take to reach the nearest motorable road from your house?

..... Minutes/Hours/Days

Appendix 3

Understanding livestock movements for the study of epidemiology of FMD in Bhutan.

Questionnaire to be administered to the village headman

District: Geog: Village:

Date: Name of respondent

Interviewer:

Section 1 Information on sale of livestock (Cattle, Sheep, Goats and Pigs)

1. Do the farmers in your geog sell livestock to other geogs/districts?

Yes [] No [] Don't know []

If No or Don't know, please go to section 2

If Yes,

1.1 How are animals sold? (*Please tick all that apply*)

- a) Directly to the buyer []
- b) Through a livestock trader []
- c) Others (please specify)

1.2 How are the sold animals taken to other geogs/districts?

- a) By walking []
- b) By vehicle []
- c) By both means []

1.3 List the geogs/districts to which farmers from your geog normally sell animals to

.....

1.4 What are the minimum, usual and maximum number of animals sold from your geog in a month?

a) Cattle

i) Minimum Number..... ii) Most likely number iii) Maximum number

b) Sheep and Goats

i) Minimum Number..... ii) Most likely number iii) Maximum number

c) Pigs

i) Minimum Number..... ii) Most likely number iii) Maximum number.....

1.5 On average, how many times in a year do farmers in your geog sell animals? (*Please tick or write*)

a) Once a year

b) Twice a year

c) Three times a year

d) Other (please specify)

.....

1.6 In which months of the year do the majority of farmers sell their animals?

.....

Section 2 Information on purchase of animals

2. Do the farmers in your geog buy animals from other geogs/districts?

Yes [] No [] Don't know []

If No or Don't know, go to Section 3

If Yes,

2.1 How do they buy the animals?

- a) Directly by contact with the seller []
- b) Through a livestock trader []
- c) Others (please specify).....

2.2 How are the animals taken to your geog?

- a) By walking []
- b) By vehicle []

2.3 List the geogs/districts from where animals are usually purchased.

.....

2.4 What are the minimum, usual and maximum number of animals purchased in your geog each month which originate from other geogs/districts?

a) Cattle

- i) Minimum Number..... ii) Most likely number iii) Maximum number

b) Sheep and Goats

- i) Minimum Number..... ii) Most likely number iii) Maximum number

c) Pigs

- i) Minimum Number..... ii) Most likely number iii) Maximum number

2.5 On average, how many times in a year do farmers in your geog buy animals?

Once a year

Twice a year

Three times a year

Other (please specify)

.....

2.7 In which months of the year do most farmers buy animals?

.....

Section 3 Information on Livestock traders operating in the geogs
--

3. Are you aware of any persons/traders involved in buying or selling animals in your Geog?

Yes [] No [] Don't know []

If No or Don't know, go to section 4

If Yes,

3.1 What species of animals do they buy from your Geog? *(Please tick all that apply)*

Cattle [] Sheep and Goats [] Pigs []

Other

3.2 Where do they take these animals to? *(Please list the destination geogs or districts)*

.....

3.3 What happens to these animals?

a) Taken to another district for slaughter []

b) Taken to another district for sale to other farmers for breeding purposes []

c) Slaughtered within the same district and the meat was sold []

d) Other (please specify)

.....

3.4 What species of animals are sold by these traders?

Cattle [] Sheep and Goats [] Pigs []

Other

3.5 List the districts from where animals are brought from?

.....

4. Do the farmers in your geog slaughter animals?

Yes [] No [] Don't Know []

If No or Don't Know, go to next section

If Yes,

a) What species of animals are slaughtered?

- (i) Cattle [] (iii) Pigs []
(ii) Sheep and goats [] (iv) Other

b) Where are the animals usually slaughtered?

.....

6. Are you aware of any cattle migration occurring within your geog? (*Cattle migration here is defined as the seasonal movement of cattle for grazing purposes and not for sale*)

Yes [] No [] Don't know []

If Yes,

6.1 Please list the geogs through which these cattle move?

.....

6.2 Where is the final destination of the migrating cattle? (Please list the districts and geogs)

.....

Appendix 4

Understanding livestock movement for the study of epidemiology of FMD in Bhutan

Questionnaire for livestock traders

District: Geog: Village:

Date: Name of respondent Sex:

Interviewer:

1. Background information of Traders

1.1 In which district are you based?

.....

1.2 Are you:

- a) A full-time trader []
- b) A regular, part-time trader []
- c) An occasional (opportunistic) trader []

1.3 Do you currently own livestock? Yes [] No []

1.4 How many years have you been involved in the livestock trade?Years.

2. Livestock trading practices

2.1 What type of livestock trade are you engaged in? *(Please tick all that apply)*

- a) Animals are bought for slaughter and the meat sold []
- b) Animals are bought to be on-sold to other farmers []
- c) Animals are bought to be resold to other trader/agents []

2.2 How many animals do you trade each year?

a) Cattle

i) Minimum Number..... ii) Most likely number iii) Maximum number

b) Sheep and Goats

i) Minimum Number..... ii) Most likely number iii) Maximum number.....

c) Pigs

i) Minimum Number..... ii) Most likely number iii) Maximum number

2.3 Can you list the Geogs/districts from where you usually buy your animals?

.....
.....

2.4 Who do you normally sell your animals to?

Butchers []

Farmers []

Other traders []

Slaughter animals and only sell the meat []

Other please specify

.....

2.5 If you sell animals which Geogs/districts are they sold?

.....

2.6 How do you *transport* animals after you have buy them?

a) By Walking []

b) By motorcycle []

c) By truck []

d) Other (Please specify):

2.7 Do you ever hold animals after buying them until you find a buyer for them?

Yes [] No [] Sometimes []

If yes, where do you hold them?

.....

2.8 Which month(s) do you most frequently trade livestock?.

Month(s)	Why?
Cattle
Pigs
Others

2.9 Which month(s) do you trade livestock the least?

Month(s)	Why?
Cattle
Pigs
Others

3. Livestock prices

3.1 What in your opinion determines the price of livestock in Bhutan?

Please rate the following factors according to importance by placing a number (0-3) in each square of the table where:

- 0 least important factor
- 1 less important factor
- 2 important factor
- 3 very important factor

Factor	Importance
Demand within Bhutan	
Time of year	
Body condition of animals being sold	
Health status of animal: whether sick or healthy	
Disease risk: whether a contagious disease is present in the village	
Festival seasons	

3.2 How do you keep track of livestock prices in different parts of Bhutan and in India?

.....

3.3 What are the minimum, average and maximum prices you usually pay for each adult animal?

Species	Category	minimum price	Most likely price	Maximum price
Cattle	Adult	Nu.....	Nu	Nu
Sheep	Adult	Nu.....	Nu	Nu
Goats	Adult	Nu.....	Nu	Nu
Pigs	Adult	Nu.....	Nu	Nu

4. Trading of diseased animals

4.1 Do farmers ever sell livestock that are sick?

Yes [] No [] Don't know []

4.2 Are you aware of the disease called 'Foot-and-mouth Disease' (FMD)?

Yes [] No [] Don't know []

4.3 Would you recognise FMD in an infected animal?

Yes [] No []

If 'Yes', how would you recognise it?

.....
.....

4.4 Can you sell an animal that was visibly sick with FMD?

Yes [] No []

4.5 Is the price much lower for an animal:

That is visibly infected with FMD? Yes [] No []

That has recovered from FMD? Yes [] No []

4.6 What would you do if an animal that you had bought was sick with FMD?

	Yes	No	Sometimes
Re-sell immediately	[]	[]	[]
Re-sell after waiting until it has recovered?	[]	[]	[]
Sell immediately to slaughterhouse	[]	[]	[]
Other (please specify)	[]	[]	[]

5. Movement regulations

5.1 Whose approval do you need to trade animals?

.....
.....

5.2 Are the movement regulations reasonable?

Yes [] No []

If not, why not?

.....
.....

Appendix 5

Questionnaire survey for disease freedom study in Tsirang

District: Geog: Village:

Date: Name of respondent

Interviewer:

Can the farmer diagnose the disease? Yes [] No []

1. Livestock population profile (Write the total numbers of animals in the relevant box)

2.1 Cattle	<input type="text"/>	2.2 Buffalo	<input type="text"/>	2.3 Sheep	<input type="text"/>
2.4 Goats	<input type="text"/>	2.5 Pigs	<input type="text"/>	2.6 Horses	<input type="text"/>
2.7 Poultry	<input type="text"/>				

2. Have you had an outbreak of FMD in your herd within the last 5 years?

Yes [] No [] Can't remember []

3. How do you replace your cattle stock?

- i) Produce from my own herd []
- ii) Buy from within the same District []
- iii) Buy from another District []
- iv) Buy from India []
- v) Don't replace at all []
- vi) Other (*please specify*)

4. Did you consume meat at home during the last one year? Yes [] No []

If No, go to section 5

If Yes,

4.1 What type of meat did you eat? *(Please tick all that apply)*

- (a) Beef []
- (b) Goat Meat []
- (c) Sheep Meat []
- (d) Pork []
- (e) Chicken []
- (f) Other (please specify)

4.2 Where did you get your meat from? *(Please tick all that apply)*

- (a) Own production/slaughter []
- (b) Buy from within my village []
- (c) Buy from a nearby town/Meat Stall []
- (d) Buy from another district []
- (e) Other (please specify)

5. Are these animals (Cattle, sheep and goats currently available in the farmer's herd) all born within this herd or brought from another area?

- (a) All were born in the herd []
- (b) Some were born within the herd and some were bought from another place []
- (c) All were bought from another place []

6. During the last five years, have you purchased cattle from another district?

Yes [] No [] Can't remember []

If No or Can't remember, go to section 7

If Yes,

6.1 List the districts from where you bought cattle from?

.....

6.2 How many times do you buy cattle each year?

- a) Once a year
- b) Twice a year
- c) Thrice a year
- d) Other (please specify)

7. Are you aware of any persons/agency involved in buying animals from your village/Geog?

Yes [] No [] Don't know []

If No or Don't know, go to section 8

If Yes,

7.1 What species of animals do they buy from your village/Geog?

Cattle [] Sheep and Goats [] Pigs []
Others.....

7.2 Where do they take these animals? (Please list the geogs or districts)

.....

7.3 Why do they buy these animals?

- a) Take to another district for slaughter []
- b) Take to another district for sale to other farmers for breeding purpose []
- c) Slaughter in Tsirang and sell the meat []
- d) Other (please specify)

.....

.....

8. Are you aware of any persons/agency involved in selling animals in your village/Geog?

Yes [] No [] Don't know []

If No or Don't know, go to section 9

If Yes,

8.1 What species of animals do they sell?

Cattle [] Sheep and Goats [] Pigs []

Other (please specify)

8.2 List the districts from where they bring the animals from

.....

9. Are you aware of any slaughter house available in your village/geog/district?

Yes [] No [] Don't know []

If No or Don't know, Go to section 10

If Yes,

a) What species of animals are slaughtered?

(i) Cattle [] (iii) Pigs []
(ii) Sheep and goats [] (iv) Poultry []

10. Are you aware of any cattle migration occurring within your village/geog/district?

Yes [] No [] Don't know []

If Yes,

10.1 Please list the geogs through which these cattle move about?

.....

10.2 Where is the final destination of the cattle migration? (Please list the district and geog)

.....

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