NATIONAL
LUMPY SKIN DISEASE
PREVENTION AND CONTROL PLAN 2021
BHUTAN

National Centre for Animal Health
Department of Livestock
Ministry of Agriculture & Forests
Royal Government of Bhutan

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FOREWORD

Lumpy skin disease (LSD) is a viral disease of cattle and possibly Asian domestic buffaloes (*Bubalus bubalis*). It is caused by lumpy skin disease virus (LSDV). The disease is characterized by fever and multiple well circumscribed, firm and deep-seated skin nodules and necrotic plaques in the mucous membranes, primarily the upper respiratory tract and oral cavity. Other common lesions include mastitis, orchitis and swelling of peripheral lymph nodes.

The disease is of huge socio-economic importance because of its prolonged debilitating effects including reduced weight-gain, temporary or permanent cessation of milk production, sometimes accompanied by mastitis, temporary or permanent infertility or even sterility in bulls because of orchitis, as well as permanent skin damage.

The first widespread outbreak of LSD was reported from Zambia in 1929. Ever since, the disease has spread to various parts of the world including Africa, Europe, the Middle East and Asia. Since mid-2019, LSD outbreaks were reported from south Asian countries. The first outbreak of LSD in Bhutan was reported in September 2020, wherein a total of 160 cattle were affected in two southern districts sharing border with India.

In response to the outbreak, the National Lumpy Skin Disease Prevention and Control Plan has been developed to guide field professionals and relevant stakeholders during implementation of LSD prevention and control measures.

I would like to thank the National Centre for Animal Health and Animal Health Division, Department of Livestock, for taking the lead in developing this document. I also extend my appreciation to all individuals who have contributed towards producing this important national plan document.

I hope this document will serve as ready reference to all the stakeholders involved in the prevention and control of LSD outbreak(s) in the country. I am confident that this document will directly contribute to preventing the incursion of LSDV into the country and control further spread during outbreak(s).

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ABBREVIATIONS AND ACRONYMS

AHD	Animal Health Division
BAFRA	Bhutan Agriculture and Food Regulatory Authority
DoFPS	Department of Forests and Park Services
DoL	Department of Livestock
DVH	Dzongkhag Veterinary Hospital
Dzongkhag	District, an administrative division comprising of a group of Gewogs in Bhutan
FAO	Food and Agriculture Organization
Gewog	Sub-district, an administrative division comprising of a group of villages in Bhutan
LEC	Livestock Extension Centre
LSDV	Lumpy skin disease virus
Mangmi/ Tshogpa	Elected block representative/leader
MoAF	Ministry of Agriculture and Forests
NCAH	National Centre for Animal Health
NLSDPCP	National Lumpy Skin Disease Prevention and Control Plan
OIE	World Organisation for Animal Health
RLDC	Regional Livestock Development Centre
RRT	Rapid Response Team
RT-PCR	Real-time Polymerase Chain Reaction
SOP	Standard Operating Procedure
Tsamdro	Bhutanese term for grazing areas including pastureland, meadows or rangelands in general. Areas registered as tsamdro are in government owned forests
Tshethar	Practice of freeing animals from imminent slaughter and death
TVH&SL	Thromde (City) Veterinary Hospital and Satellite Laboratory
VO	Veterinary Officer
VPP	Veterinary paraprofessional

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1. BACKGROUND

Lumpy skin disease (LSD) is an infectious disease of cattle caused by the LSD virus of genus *Capripoxvirus* and family Poxviridae. The transmission occurs mainly mechanically by arthropod vectors, indirectly via contaminated feed and water, iatrogenically via semen and potentially through direct contact. It was also suggested that hard ticks might be involved in LSD virus transmission. LSD is listed as notifiable by the OIE (World Organisation for Animal Health) due to its potential for rapid spread and substantial economic impact, causing reduction in milk production, decreased growth rate in beef cattle, temporary or permanent sterility in bulls, damage to hides and abortion.

In south and south-east Asia, since mid-2019, Lumpy skin disease outbreaks were reported from China (People's Republic of), Chinese Taipei, Bangladesh, and India. On 29 August 2020, Nepal also reported outbreaks of LSD to OIE. The outbreak of LSD in Bhutan was, for the first time, confirmed on 5 October 2020 after testing (RT-PCR) the samples of suspected cases received from Samtse district, a district in the southwest sharing border with the Indian state of West Bengal.

The National Lumpy Skin Disease Prevention and Control Plan for Bhutan is being developed in response to this outbreak and considering other risk factors for incursion and spread of the LSD virus in Bhutan. The document shall guide field professionals and relevant stakeholders for the effective implementation of LSD prevention and control measures. The measures outlined in the document shall undergo periodic testing through simulation exercises and updated from time to time as per the need.

1.1. Goal and objective

The overall goal of this document is to prevent and control the LSD outbreak in Bhutan and eventually eliminate it. Achieving this will contribute to the upliftment of rural socio-economic status through improved livestock health and production, and trading.

The objectives of this plan document are to inform policymakers and stakeholders on the nature and purposes of the LSD prevention and control programmes at national, regional and district levels; and to provide field professionals and relevant stakeholders with strategic directions to prevent, control and progressively decrease the outbreak incidences. This document shall also guide the implementation of LSD prevention and control measures by strengthening laboratory diagnostic capacity, epidemiological capacity, prevention strategy, biosecurity measures, and legal frameworks. The progress in the implementation phases shall be assessed through regular monitoring and evaluation processes.

2. GENERAL DESCRIPTION OF THE DISEASE

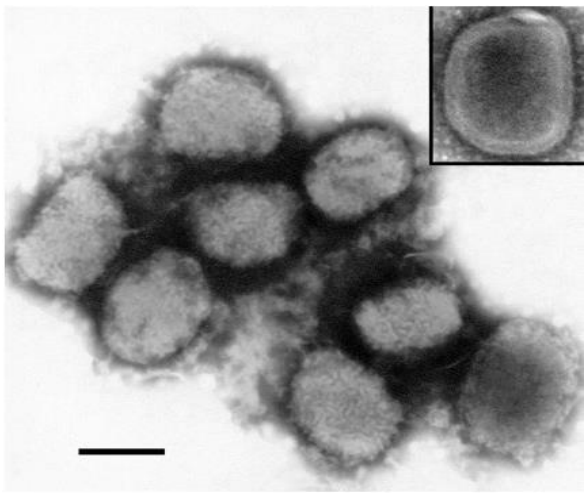
2.1. Aetiology

2.1.1. The virus

Lumpy skin disease virus (LSDV) is a member of the genus *Capripoxvirus* within the family Poxviridae (subfamily Chordopoxvirinae) (Table 1). The other members of the genus include the sheeppox virus (SPPV) and the goatpox virus (GTPV). Mature capripox virions (Figure 1) have a similar morphology to those of orthopoxviruses (prototype, vaccinia virus) when viewed using transmission electron microscopy, although capripox virions tend to be less symmetrical in shape, 120 with average dimensions of 320 × 260 nm, giving an axis ratio of approximately 1:2.88. A cross-sectional analysis of the virions reveals a pair of lateral bodies (Figure 2), common to all poxviruses. Compared to other poxviruses, the double-stranded DNA genomes of capripoxviruses are relatively small, averaging around 150 kilo-base pairs (kbp) (compared with avipoxvirus genomes of around 300 kbp). Their genomes are highly conserved between the three genus members, with LSDV containing two unique genes compared to sheeppox or goatpox viruses. The viruses share around 97 percent sequence identity. Centrally located "house-keeping" genes (coding for structural proteins, proteins involved in replication, transcription etc.) are the most highly conserved, in line with other poxviruses, with the inclusion of less conserved "virulence" genes (coding for proteins involved in pathogenesis) towards the termini, a number of which have putative immunomodulatory functionality for host immune response.

Table 1: Virus identity

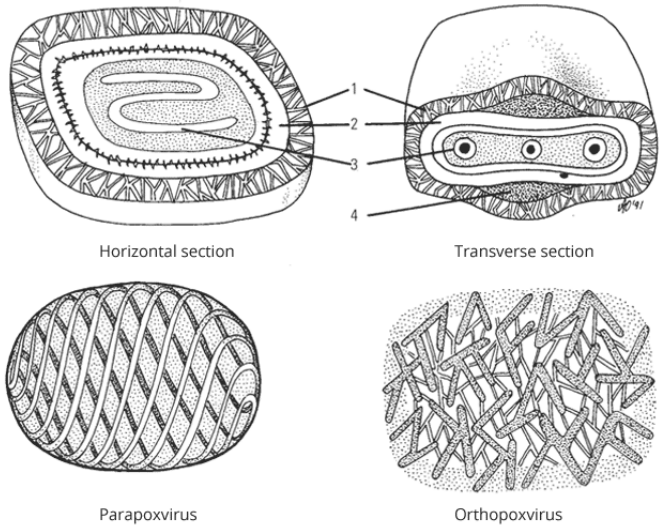
Preferred scientific name Lumpy skin disease virus
International common names English: Neethling virus
English acronym LSDV
Taxonomic Tree Domain: Virus Group: "ssDNA viruses" Group: "DNA viruses" Family: Poxviridae Subfamily: Chordopoxvirinae Genus: Capripoxvirus Species: lumpy skin disease virus



Picture courtesy: F. Glyn Davies

Figure 1: Electron micrograph of LSD virus particles

Available evidence suggests that there is only one serotype of LSDV. Virus isolates collected over an extended period from many natural cases originating from outbreaks of the disease in South Africa, Kenya, and Malawi, showed reciprocal cross-neutralization with the prototype Neethling strain. Complete genome sequencing of recent LSDV isolates SERBIA/Bujanovac/2016 and Evros/GR/15 demonstrated 99.5 per cent and 99.8 per cent homology respectively with the LSDV field isolate Neethling Warmbaths LW isolated in South Africa in 2000, indicating genetic stability of LSDV as well as providing genetic evidence in support of a single serotype.



Picture courtesy:
Coetzer et al., 2018.

Figure 2: Line drawings of poxviruses.

1 = double membrane with filaments; 2 = surface protein; 3 = core

2.1.2. Sources of virus

The sources of LSDV are as follows:

- Skin nodules, scabs and crusts which contain relatively high amounts of LSDV. The virus can be isolated from this material for up to 35 days and likely for longer.
- LSDV can be isolated from blood, saliva, ocular and nasal discharge, and semen.
- LSDV is found in the blood (viraemia) intermittently from approximately 7 to 21 days post-infection at lower levels than present in skin nodules.
- Shedding in semen may be prolonged; LSDV has been isolated from the semen of an experimentally infected bull 42 days post-inoculation.
- There has been one reported case of placental transmission of LSD.

2.1.3. Persistence of virus

Persistence or vulnerability of lumpy skin disease virus (LSDV) to various physical and chemical agents are as described in the following table.

Table 2: Susceptibility of LSDV to physical and chemical agents

Temperature	Susceptible to 55°C for 2 hours, or 65°C for 30 minutes. It can be recovered from skin nodules kept at –80°C for 10 years and infected tissue culture fluid stored at 4°C for 6 months.
pH	Susceptible to alkaline or acidic pH. No significant reduction in titre when held at pH 6.6–8.6 for 5 days at 37°C.
Chemicals/Disinfectants	Susceptible to ether (20%), chloroform, formalin (1%), and some detergents, e.g., sodium dodecyl sulphate. Susceptible to phenol (2% for 15 minutes), sodium hypochlorite (2–3%), iodine compounds (1:33 dilution), Virkon® (2%), quarternary ammonium compounds (0.5%).
Survival	LSDV is remarkably stable, surviving for long periods at ambient temperature, especially in dried scabs. LSDV is very resistant to inactivation, surviving in necrotic skin nodules for up to 33 days or longer, desiccated crusts for up to 35 days, and at least 18 days in air-dried hides. It can remain viable for long periods in the environment. The virus is susceptible to sunlight and detergents containing lipid solvents, but in dark environmental conditions, such as contaminated animal sheds, it can persist for many months.

2.2. Disease epidemiology

For Lumpy skin disease, the morbidity rate varies between 10 and 20% and the mortality rates of 1 to 5% are considered usual.

2.2.1. Susceptible host

LSDV is highly host-specific and causes diseases only in cattle (*Bos indicus and B. taurus*) and water buffalo (*Bubalus bubalis*). There is evidence from a study in Ethiopia of differential breed susceptibility to LSD, with Holstein Friesian or crossbred cattle exhibiting higher morbidity and mortality due to LSD when compared with local zebu cattle.

Some LSDV strains may replicate in sheep and goats. Although mixed herds of cattle, sheep and goats are common, to date no epidemiological evidence on the role of small ruminants as a reservoir for LSDV has been reported. Clinical signs of LSD have been demonstrated after experimental infection in impala (*Aepyceros melampus*) and giraffe (*Giraffa camelopardalis*). The disease has also been reported in an Arabian oryx (*Oryx leucoryx*) and springbok (*Antidorcas marsupialis*). Extensive serological surveys of wild ruminant species in Africa have not identified a wildlife reservoir of LSDV.

LSDV is not zoonotic.

2.2.2. Transmission

The first case of LSD can often be traced to the legal or illegal transfer of cattle between farms, regions or even countries. Movements of cattle may allow the virus to jump over long distances. Short-distance leaps, equivalent to how far insects can fly (usually < 50 km), are occasioned by numerous local blood-feeding insect vectors feeding on cattle and changing hosts frequently between feeds. No evidence exists of the multiplication of the virus in vectors, but it cannot be excluded. The principal vector is likely to vary between geographical regions and ecosystems. The common stable fly (*Stomoxys calcitrans*), the *Aedes aegypti* mosquito, and some African tick species of the *Rhipicephalus* and *Amblyomma* spp., have demonstrated the ability to spread the LSDV. Viral transmission from infected carcasses to naïve live animals via insects is a possible risk but has not been sufficiently studied.

Direct contact is considered ineffective as a source of infection but may occur. Infected animals may be viraemic only for a few days, but in severe cases, viraemia may last for up to two weeks. Infected animals showing lesions in the skin and mucous membranes of the mouth and nasal cavities excrete infectious LSDV in saliva, as well as in nasal and ocular discharges, which may contaminate shared feeding and drinking sites. To date, infectious LSDV has been detected in saliva and nasal discharge for up to 18 days post-infection. More research is needed to investigate how long the infectious virus is excreted in such discharge. Infectious LSDV remains well-protected inside crusts, particularly when these drop off from the skin lesions. Although no experimental data are available, it is likely that the natural or farm environments remain contaminated for a long time without thorough cleaning and disinfection. Field experience shows that when naïve cattle are introduced to LSDV-infected holdings after stamping out, they become infected within a week or two – indicating that the virus persists either in vectors, the environment, or both.

The virus persists in the semen of infected bulls so that natural mating or artificial insemination may be a source of infection for females. Infected pregnant cows are known to

deliver calves with skin lesions. The virus may be transmitted to suckling calves through infected milk, or from skin lesions in the teats.
Iatrogenic intra- or inter-herd transmission may occur via contaminated needles during vaccination or other injections if needles are not changed between animals or herds. Eventually, affected animals clear the infection and there is no known carrier state for LSDV.

Source: FAO, 2017

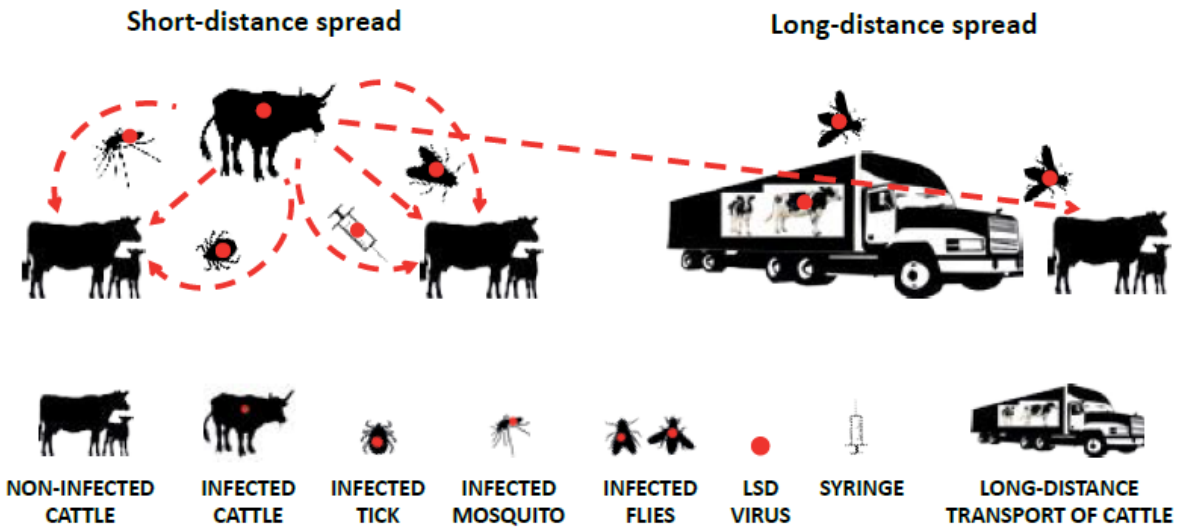


Figure 3: Schematic illustration of the spread of LSDV

2.2.3. Geographic distribution

The disease made its first emergence in Zambia in 1929 and later spread to the whole African continent except Libya, Algeria, Morocco and Tunisia. With the increasing demand for food, Middle east countries have increased the transportation of animals from neighbouring countries. LSD infection in Egypt in 1988 was due to the movement of infected cattle from affected African countries. In 2006 again, the disease re-emerged due to unrestricted movement of cattle from African horn countries.
The first outbreak in Israel in 1989 was thought to be due to the movement of infected *Stomoxy calcitrans* from Egypt. From 2012 to 2013 the disease appeared for the first time in Syria, Jordan, and Lebanon. The outbreak in Jordan appeared near the border of Israel and Syria indicating the transboundary spread of disease. The disease further spread to other nearby countries like Turkey and Iraq in 2013 and Iran in 2014. Later, LSD was also reported from Cyprus, Azerbaijan, and Turkey. From the OIE report, LSD has been re-emerged in Israel after 6 years in 2019 due to a decrease in vaccination of animals, which was earlier mandatory for the animals. In 2019, diseases have been re-emerged in the southern province

of Turkey and the eastern province of Russia. Figure 4 shows the spread of LSDV from the place of its first emergence to other parts of the world.

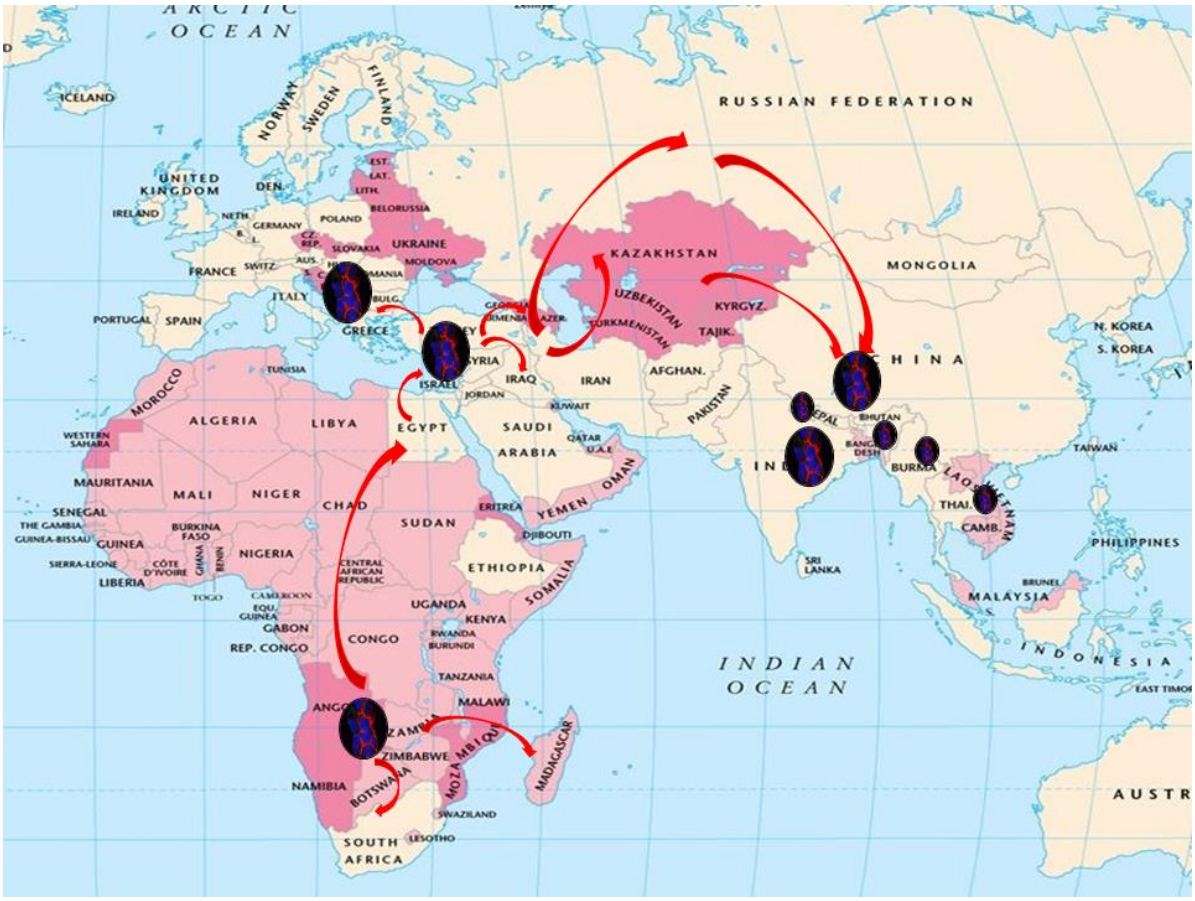


Figure 4: Illustration of the spread of LSDV in the world.

For the first time, LSD outbreaks were reported from Bangladesh, India and China in 2019; Chinese Taipei, Vietnam, Bhutan, Hong Kong (SAR-PRC) and Nepal in 2020; and Sri Lanka in 2021 (Figure 5). In India, the first outbreak of the disease was reported in Odisha state in August 2019. In the first published report of LSD in India, it was found that out of 2,539 animals, 182 were positive with no mortality but 7.1% morbidity. Based on phylogenetic analysis, the strain present in India was genetically close to South African NI2490/KSGP-like strains rather than European strains.

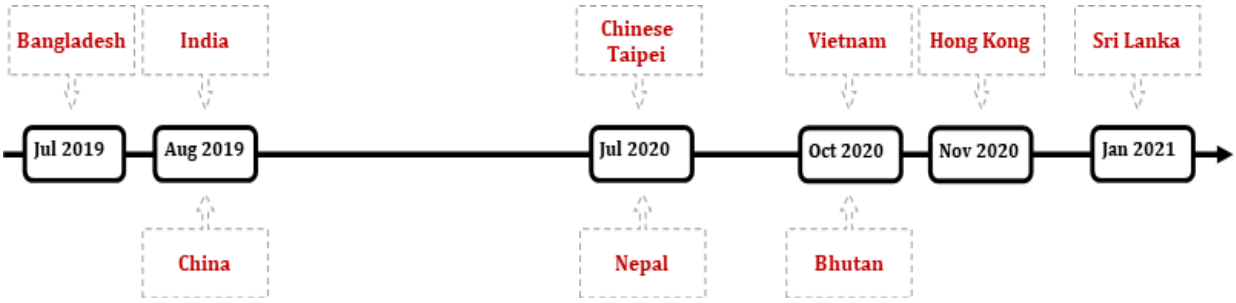


Figure 5: Timeline for recent LSD outbreaks in Asia and the Pacific.

Currently, Lumpy skin disease is endemic in most of Africa, parts of the Middle East and Turkey.

2.3. Diagnosis

Under experimental conditions, following the virus inoculation, the incubation period is between 4 and 14 days. For the *Terrestrial Manual (OIE)* purposes, the incubation period is 28 days.

2.3.1. Clinical diagnosis

Clinical signs and post-mortem lesions (Figure 7) include:

- Lachrymation and nasal discharge – usually observed first.
- Subscapular and pre-femoral lymph nodes become enlarged and are easily palpable.
- High fever (>40.50°C) may persist for approximately a week.
- A sharp drop in milk yield.
- The appearance of highly characteristic, nodular skin lesions of 10 – 50 mm in diameter:
  - The number of lesions varies from a few in mild cases to multiple lesions in severely infected animals.
  - Predilection sites are the skin of the head, neck, perineum, genitalia, udder and limbs.
  - Necrotic plaques in the mucous membranes of the oral and nasal cavities cause purulent or mucopurulent nasal discharge and excessive salivation, containing high concentrations of virus.
  - Typically, the centre of the lesion ulcerates and scab forms on top.
  - Skin nodules may persist for several months.
  - The nodules are painful and involve the epidermis, dermis, and subcutaneous tissue and may even involve the musculature. As the disease progresses, the nodules become necrotic, and eventually a deep scab forms; this lesion is called a sit fast.
- Sometimes, painful ulcerative lesions develop in the cornea of one or both eyes, leading to blindness in the worst cases.
- Skin lesions in the legs and on top of the joints may lead to deep subcutaneous infections complicated by secondary bacterial infections and lameness.

- Pneumonia caused by the virus itself or secondary bacterial infections, and mastitis are common complications.
- Subclinical infections are common in the field.
- When an animal with multiple skin lesions is sent to a slaughterhouse, sub-cutaneous lesions are visible after the animal is skinned.
- In a post-mortem examination, pox lesions can be found throughout the entire digestive and respiratory tracts and on the surface of almost any internal organ.

The following figure shows the description of the lesion development process and timeline after infection with the LSD virus.

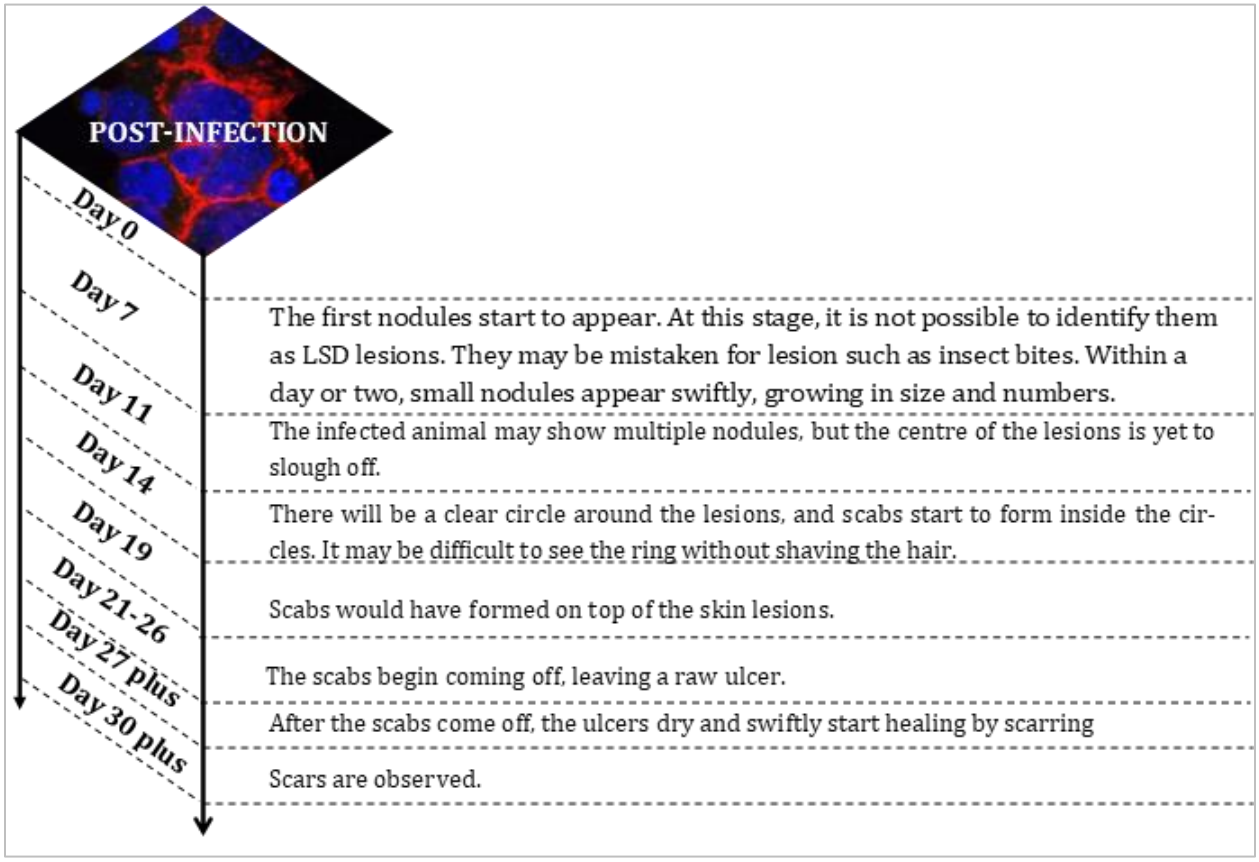


Figure 6: LSD post-infection lesions development

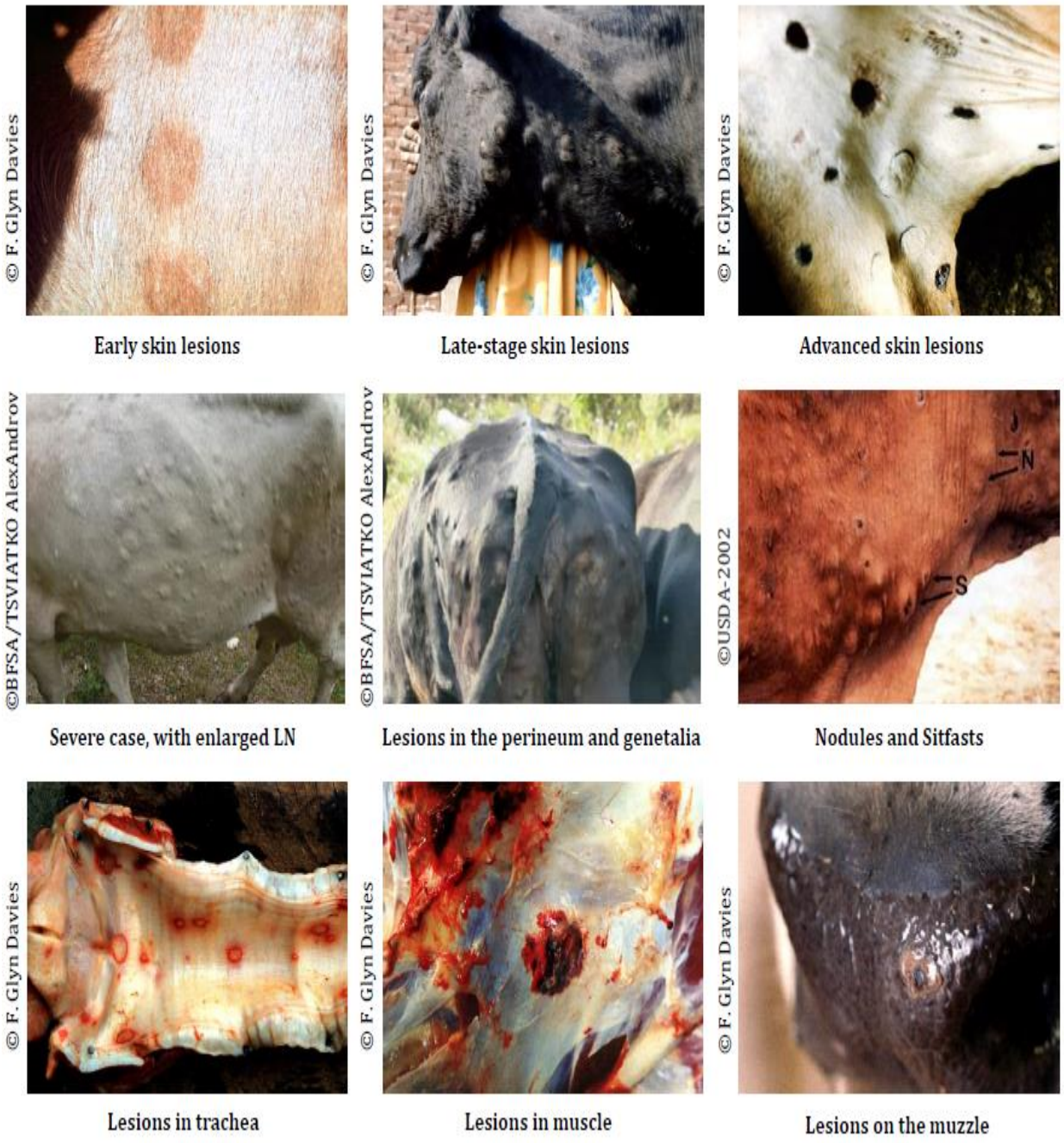


Figure 7: Clinical signs and lesions

2.3.2. Differential diagnosis

Severe cases of LSD are highly characteristic and easy to recognize. But early stages of infection and mild cases may be difficult to distinguish even for the most experienced veterinarians, requiring a laboratory confirmation. Samples should be collected from all suspected animals and tested using fast and highly sensitive PCR methods to differentiate true cases.

The following diseases may be considered as a differential diagnosis for LSD:

- Pseudo lumpy skin disease/Bovine herpes mammillitis (bovine herpes virus 2): dermal lesions may look like those caused by LSDV but are more superficial and the course of the disease is shorter and less severe. The disease can be ruled out by detecting LSDV by PCR.
- Insect bites, urticaria, and photosensitisation: dermal lesions may look like those caused by LSDV but are more superficial and the course of the disease is shorter and less severe. The disease can be ruled out by detecting LSDV by PCR.
- Pseudocowpox (Parapoxvirus): lesions occur only on the teats and udder. The disease can be ruled out by detecting LSDV by PCR.
- Dermatophilosis: early ringworm lesions, more superficial, clearly different, the non-ulcerative surface structure of the ringworm lesion.
- Demodicosis: dermal lesions predominantly over withers, neck, back, and flanks, often with alopecia present. The disease can be ruled out by the detection of mites using skin scrapings.
- Bovine papular stomatitis (Parapoxvirus): lesions occur only in the mucous membranes of the mouth. The disease can be ruled out by PCR testing.
- Besnoitiosis: lesions often occur in the scleral conjunctiva, and dermal lesions may exhibit alopecia with thick and wrinkled skin. The disease can be ruled out by detecting LSDV by PCR.
- Onchocerciasis: dermal lesions most likely at the ventral midline. The disease can be ruled out by PCR.

2.3.3. Laboratory diagnosis

The diagnosis of LSD may be tentatively made after the appearance of the typical skin lesions. Rapid and accurate laboratory confirmation of the diagnosis enables the swift implementation of appropriate measures to control the spread of the disease.

2.3.3.1. Identification of the agent

- Polymerase chain reaction (PCR) is the least expensive and quickest method for the detection of LSDV. Skin nodules and scabs, saliva, nasal secretions, and blood are suitable samples for PCR detection of LSDV.
- Virus isolation (VI) followed by PCR to confirm the virus identity takes longer and is more expensive but has the advantage of demonstrating the presence of live virus in the sample.
- Electron microscopy can be used to identify the classic poxvirus virion but cannot differentiate to genus or species level.

2.3.3.2. Serological tests

It is not possible to distinguish the three viruses in the *Capripoxvirus* genus (Sheeppox virus, Goatpox virus and LSD virus) using serological techniques.

- Virus neutralisation: this is currently the gold standard test for the detection of antibodies raised against capripoxviruses.
- Western blot: highly sensitive and specific but expensive and difficult to perform.
- Capripoxvirus antibody enzyme-linked immunosorbent assay: new commercial kits for detection of capripoxvirus antibodies are currently being developed and released in the market.

For detailed information on sample collection and diagnosis, refer to Annexure 2, SOP 5 for sample collection and SOP 7 for detection of Capripox (including LSDV) in suspected samples.

2.4. Treatment

There is no effective treatment for LSD. Supportive therapy with antibiotic and anti-inflammatory veterinary medicines practised whenever pyaemic changes or cellulitis occur in association with lesions. The secondary mastitis arising from teat or udder lesions requires antibiotic infusions or injections. Antiseptic dressing of wound lesions is also important.

2.5. Economic importance

The World Organisation for Animal Health (OIE) categorises LSD as a notifiable disease due to its economic impact. LSD has been considered an agro-terrorism agent due to its ability to spread from Africa to other parts of the world.

LSD is a disease of high economic importance. Although the mortality rate is generally low, economic losses result from loss of condition, decreased milk production, abortions, infertility, and damaged hides. In intensive cattle farming, direct and indirect production losses caused by LSD have been estimated to be as high as 45 – 65%. In developing countries, the poorest small-scale farmers and rural communities, whose livelihood is dependent on cattle, bear the heaviest burden.

Total losses on the account of milk, meat, beef, power of draft, treatment, and vaccination, in Ethiopia, were estimated to be 6.43 USD per head in local zebu and 58 USD per head for Holstein Friesian. In an outbreak in Jordan, supportive antibiotic treatment cost was estimated to be 27.9 British pounds per head.

3. SITUATIONAL ANALYSIS

3.1. LSD outbreaks in Bhutan

Samtse dzongkhag (district) reported the first suspected outbreak of the lumpy skin disease (LSD) in Bhutan in September 2020 and the cases were confirmed by RT-PCR as LSD on 5 October 2020 in the National Veterinary Laboratory (NVL) of the National Centre for Animal Health (NCAH), Department of Livestock (DoL).

On 18 September 2020, another outbreak of LSD was reported from Shompangkha gewog of Sarpang dzongkhag. In these two districts, Samtse and Sarpang (Figure 8), a total of 11 outbreaks were reported affecting 160 cattle, and 3 died of the infection (apparent CFR = 1.87%).

Source: The status of notifiable animal diseases in Bhutan 2020

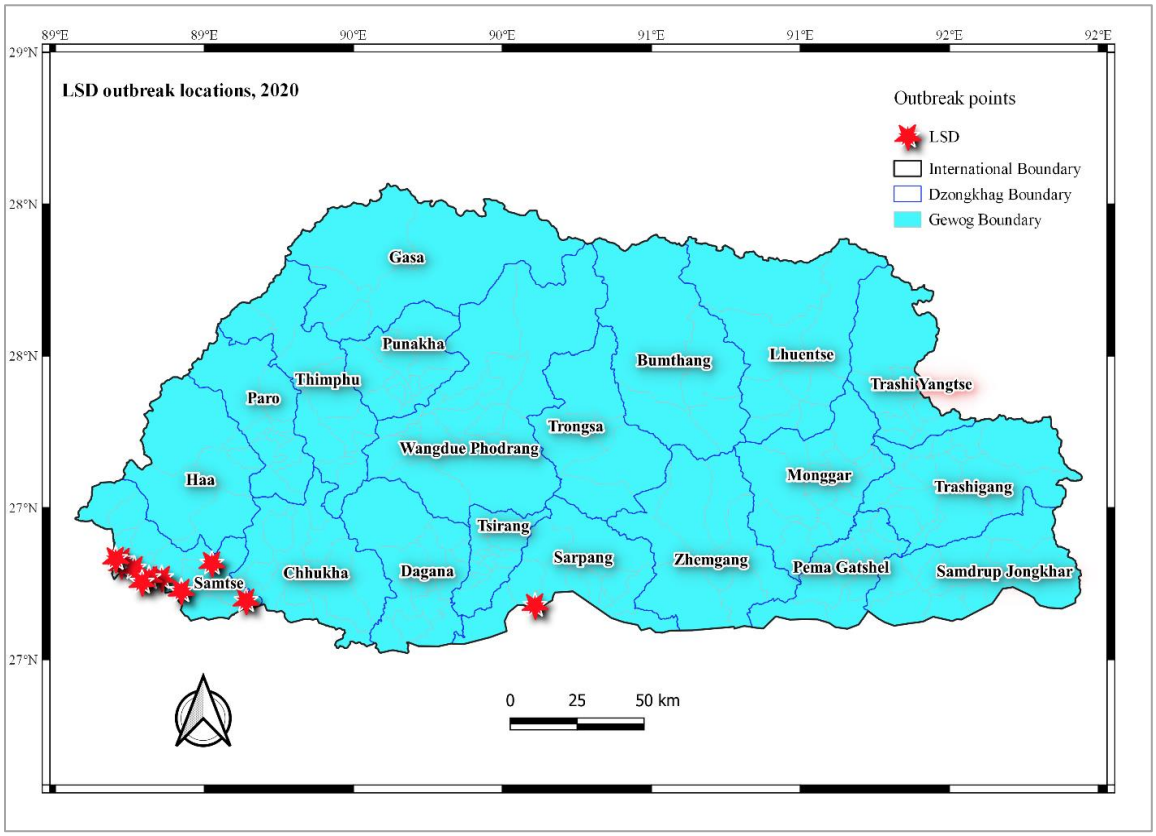


Figure 8: Location of LSD outbreaks in Bhutan, 2020

A brief descriptive analysis and the findings concerning LSD outbreaks in Samtse district are presented in the following (Data source: Disease outbreak investigation team).

- A total of 152 cattle were affected in 44 villages of 10 gewogs, and 2 of the affected animals died due to the infection (apparent CFR = 1.31 %).
- Symptomatic and supportive treatments were provided to 97 cattle (64 %).

- 57 percent of the affected cattle were adult (n=87), followed by 24 % young (n=36) and 19 % calf (n=29).
- 76 percent of the affected animals were female (n=115).
- 52 percent of the cattle affected crossbreeds, followed by 40 % native breeds and 8 % pure breeds.
- The index case date was found to be around 1 July 2020 and the last case on 20 October 2020.

3.2. Susceptible livestock population

Lumpy skin disease is primarily a disease of cattle. Most of the LSD outbreaks recorded says that exotic breeds are more susceptible than indigenous breeds. Clinical cases have also been reported in Asian water buffalo (*Bubalus bubalis*), but this species is reported to show limited susceptibility to LSD. Isolation of LSDV from skin lesions of buffalo in Egypt has been described but reported that most Asian water buffalo exposed during the 1989 Egyptian LSD epizootic did not show lesions in the field.

Data source: Annual livestock statistics for Bhutan, 2019, DoL

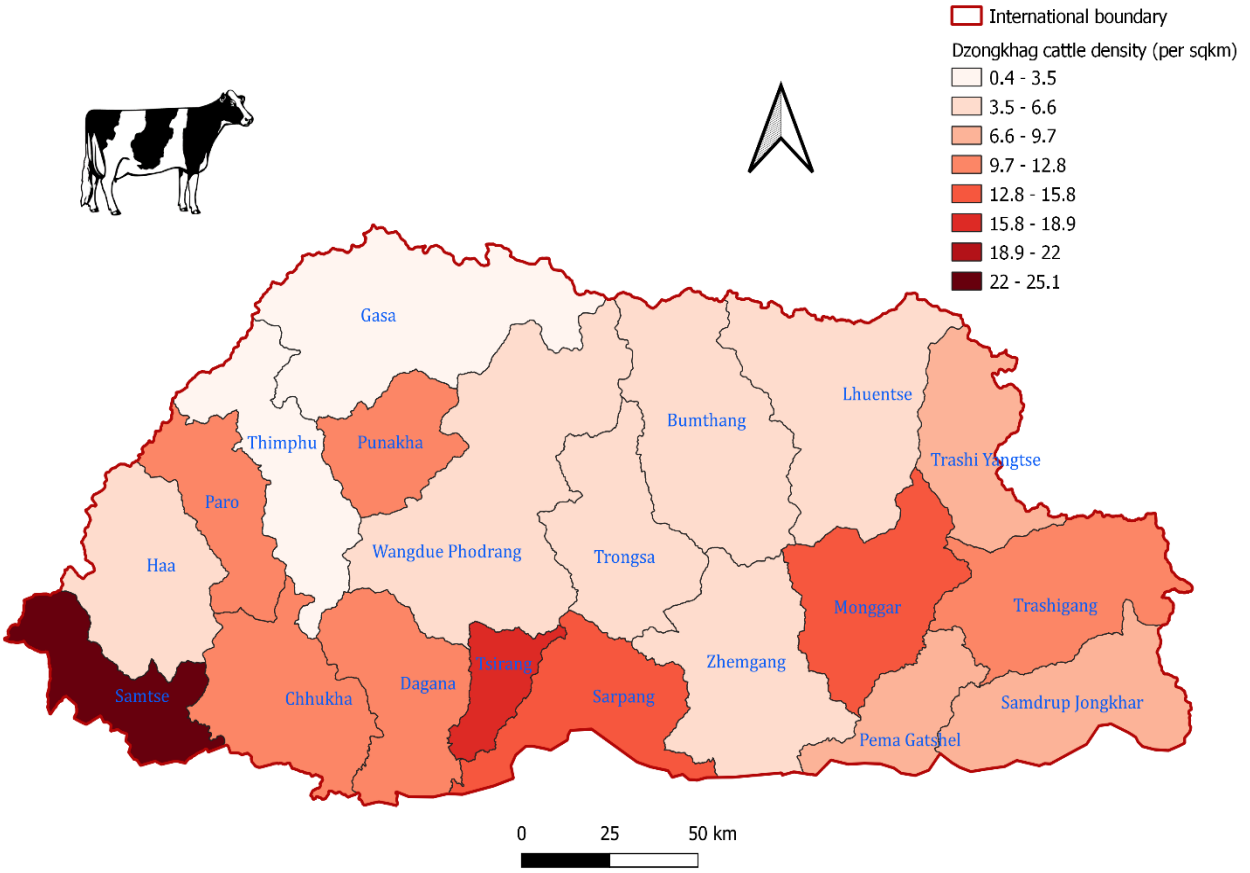


Figure 9: Cattle population density map

As per the annual livestock statistics for the calendar year 2019 published by the Department of Livestock, Ministry of Agriculture and Forests, there are 301,811 cattle and 477 buffaloes in the country. About 54 percent of the cattle population are distributed across the districts (south and east) sharing a border with India.

3.3. Cattle management practices

The cattle production systems in Bhutan are broadly categorized as transhumant and sedentary. A typical transhumant system involves the migration of cattle and herding on the traditional seasonal pastures during the summer and autumn seasons. Yak herding in the pastoral system is more common in the highland regions of the country. Forest grazing is also considered a more traditional farming practice. On the other hand, the sedentary system is defined as a crop-cattle system where each household manages few cattle in their homestead. Under this system, there is an increasing trend of providing improved housing and feeding and with improved breeds of animals.

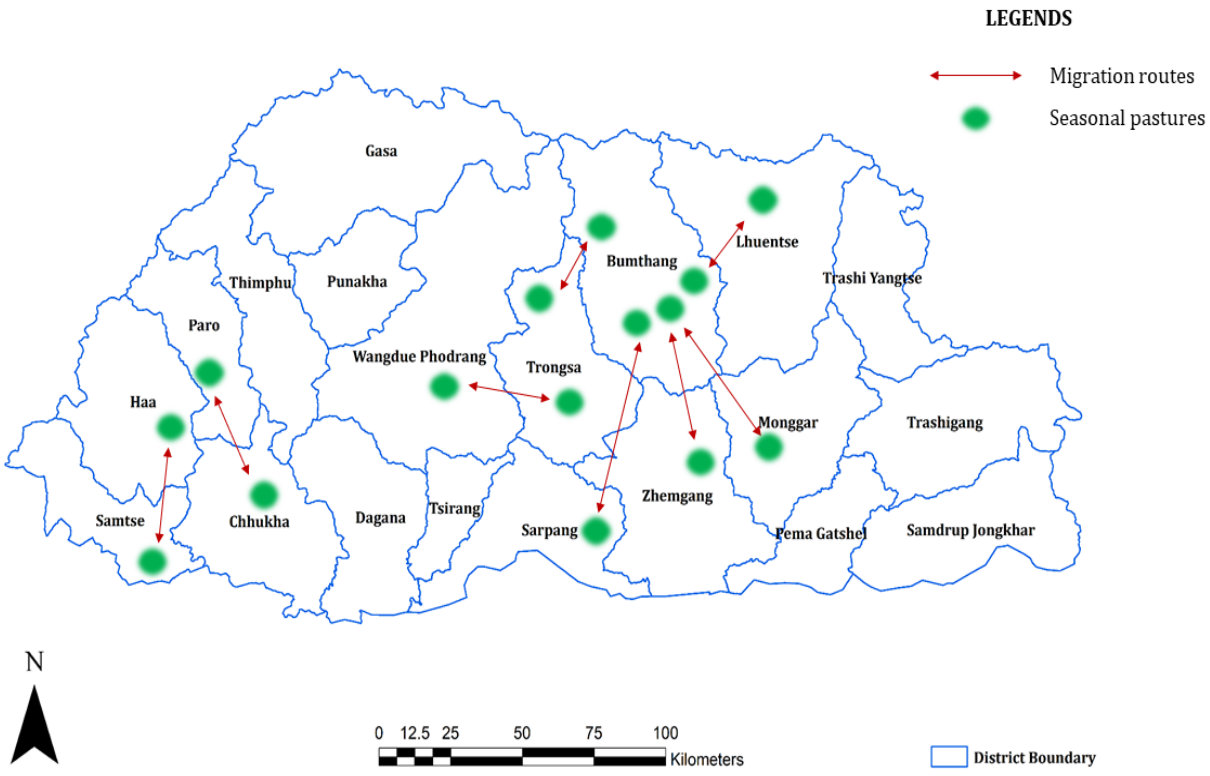


Figure 10: Cattle migration routes to seasonal pastures

Except few organized dairy farms, where stall feeding is practised, the majority of the animals are let out for free grazing in the forests during the daytime. Mixing of cattle between different herds within and between villages occur at the grazing and watering points, thus

transmitting and spreading infectious diseases is likely. There is also a mixing of animals along the border between Bhutan and India which could be an important determinant for the incursion of livestock diseases into the country. However, due to the government policy to promote improved breeds through breed intensification programme, many farmers have adopted a stall-feeding system of cattle rearing.

In the central and western part of Bhutan, the age-old tradition of seasonal migration of cattle herds persists (Figure 10). The cattle herds migrate from higher altitude areas to lowlands during September and October months and return to summer pastures from lowlands between April and June months. Seasonal migration of cattle herds is commonly practised in Paro, Haa and Bumthang districts. On average, the size of migratory herds ranges from 8 – 59 animals, and it takes 5 – 10 days for migration.

3.4. Cattle trading system

3.4.1. In-country

Data source: NDRDC, DoL

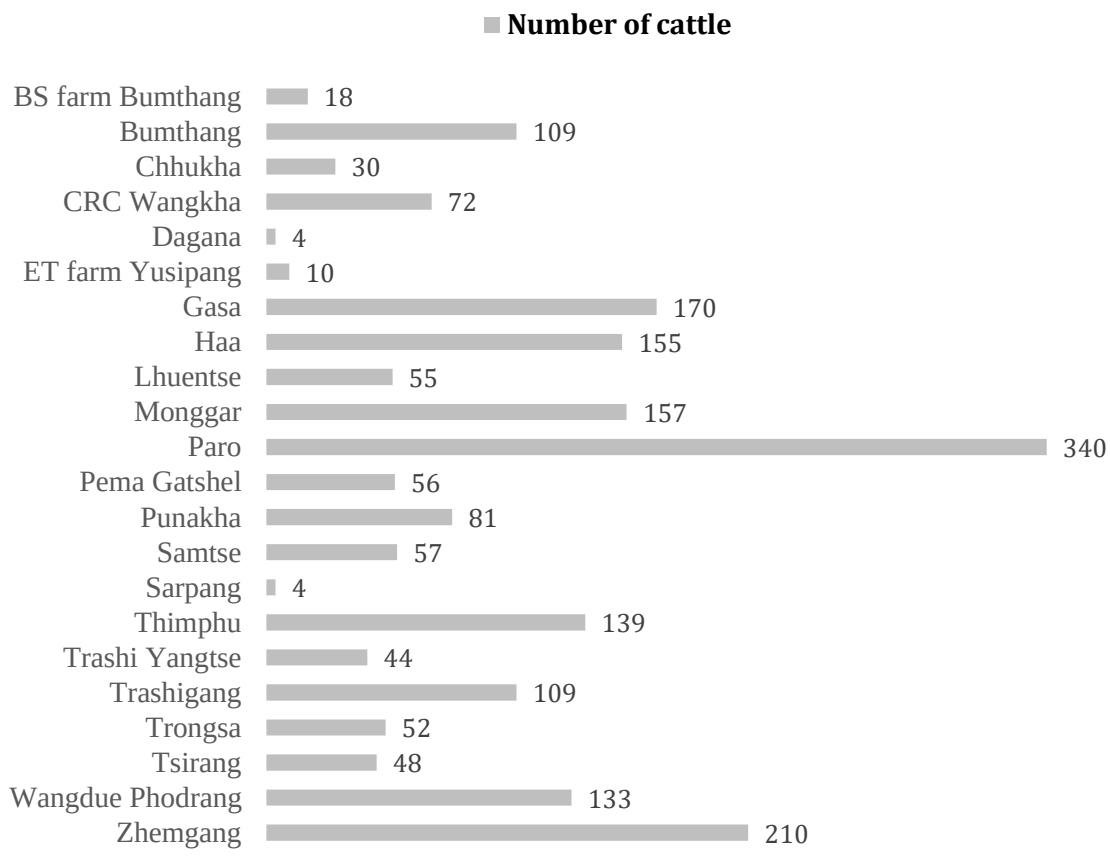


Figure 11: In-country cattle sourcing, 2008-2018

Some districts such as Samtse, Tsirang, and Sarpang act as cattle trading hub in the country. The animals are traded mainly for breeding purpose, while few trading from northern to southern districts may be for marketing across the border for slaughter.

During the 10th (2008 – 2013) and 11th (2013 – 2018) five-year plans of Bhutan, a total of 2,053 cattle were procured from within the country (Figure 11) and distributed to various Dzongkhags through government subsidy and government farms.

3.4.2. Ex-country

Ex-country sourcing of improved cattle breeds has been a practice for few decades to improve dairy production in the country. Between 2008 and 2018, a total of 2,832 cows were procured and distributed to various Dzongkhags and government farms across the country (Figure 12), worth 86.5 million Bhutanese Ngultrum. All these cattle were imported from various states of India; the North-eastern states of India being the major source.

Data source: NDRDC, DoL

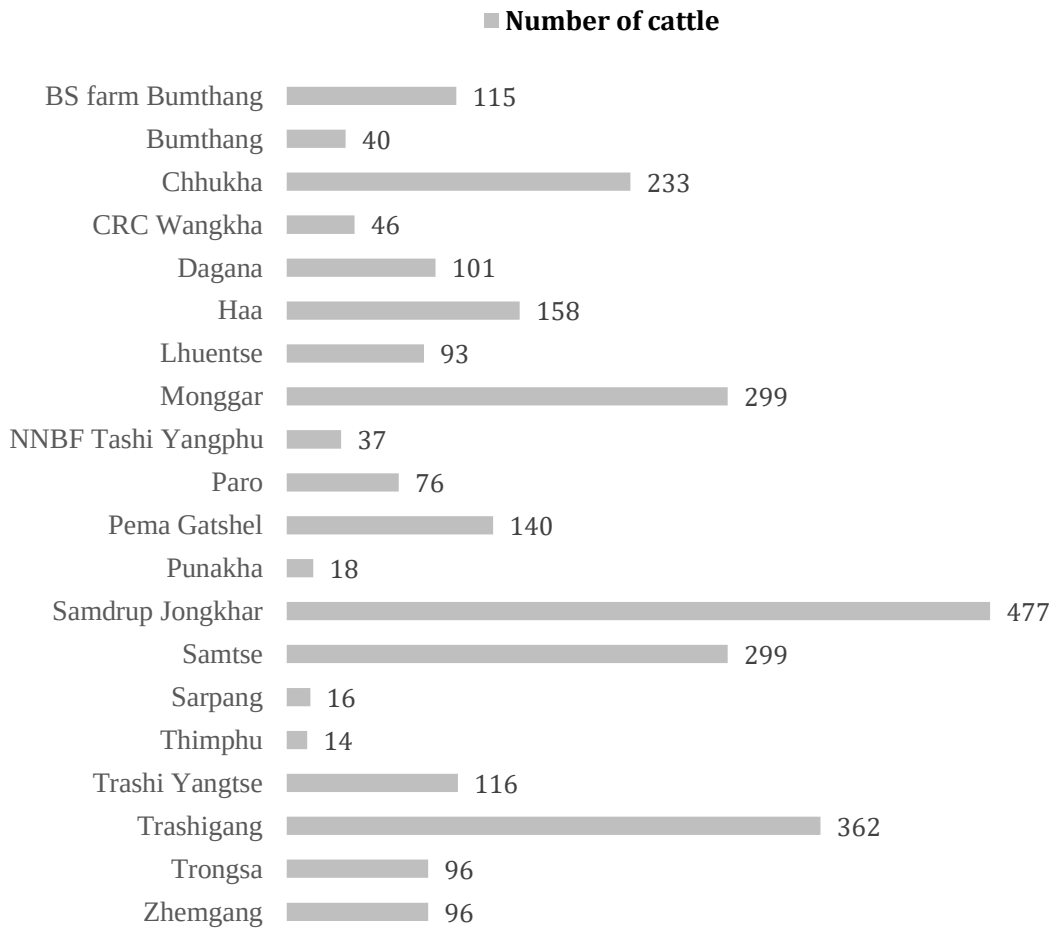


Figure 12: Ex-country cattle sourcing, 2008-2018

In addition to the import of live cattle, Bhutan continues to import dairy and meat products into the country. Meat (beef and others) import between 2010 and 2018 were consistently high, contributing to about 80% of the total consumption.
Due to people's preference over different types of value-added dairy products, the overall import figure for dairy products over the period was high, in comparison to the domestic production.

3.5. Risk assessment

3.5.1. Risk hotspots identification

To establish the risk zone, it is important to identify the risk hotspots for LSDV entry and spread in Bhutan. The identified risk hotspots are:

- Districts sharing a porous border with the north-eastern states of India.
- Quarantine stations along the southern border.
- Seasonal cattle migration practices and places.
- In-country cattle trading practices and places.

3.5.1.1. Districts bordering India

The districts sharing a border with India are the risk hotspots, as there is frequent movement of live animals and animal products across the border. The disease being vector-borne, it is very likely for the cattle along the border to get the disease from biting flies and insects carrying infection from the other side of the border.

3.5.1.2. Quarantine stations

There are four Quarantine Stations (QS) located along the India-Bhutan border: Gelegphu QS, Samdrup Jongkhar QS, Phuentshogling QS and Samtse QS. These are the main entry points for the formal import of cattle into the country (Figure 13).

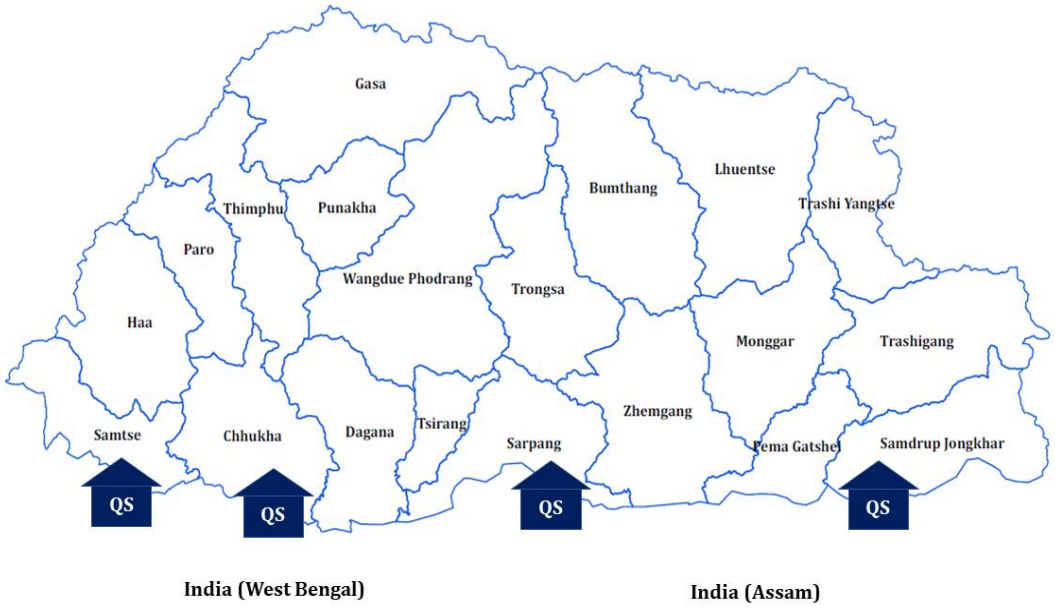


Figure 13: Animal quarantine stations in the border districts, Bhutan

3.5.1.3. Cattle migration practice

The seasonal cattle migration takes place between the summer pastures located at higher altitude areas of central and western Bhutan and winter pastures located in the southern and low-lying districts (Figure 10). Thus, the risk for incursion and spread of LSDV into interior parts of the country is very high.

3.5.1.4. In-country cattle trading

In Bhutan, Samtse and Sarpang are the major districts supplying cattle to other parts of the country. Due to the high cattle population in these districts and sharing a porous border with Indian states, these districts are critical when incursion and spread of LSDV into Bhutan is concerned.

3.5.2. Risk zones

The risk zone identification and categorization are imperative for defining the strategies in each zone and establish disease status in the country. Based on the disease epidemiology, proximity to neighbouring countries, previous outbreaks, susceptible population, and management practices, the country is divided into three risk zones: High, Medium, and Low (Figure 14).

The disease risk zones will be used in guiding the strategies for surveillance and animal movement regulation. However, the risk zones should be reviewed periodically based on the disease status to increase the efficiency of the disease prevention and control programme. Individual gewogs and dzongkhags are categorized into these three risk zones in Annexure 4.

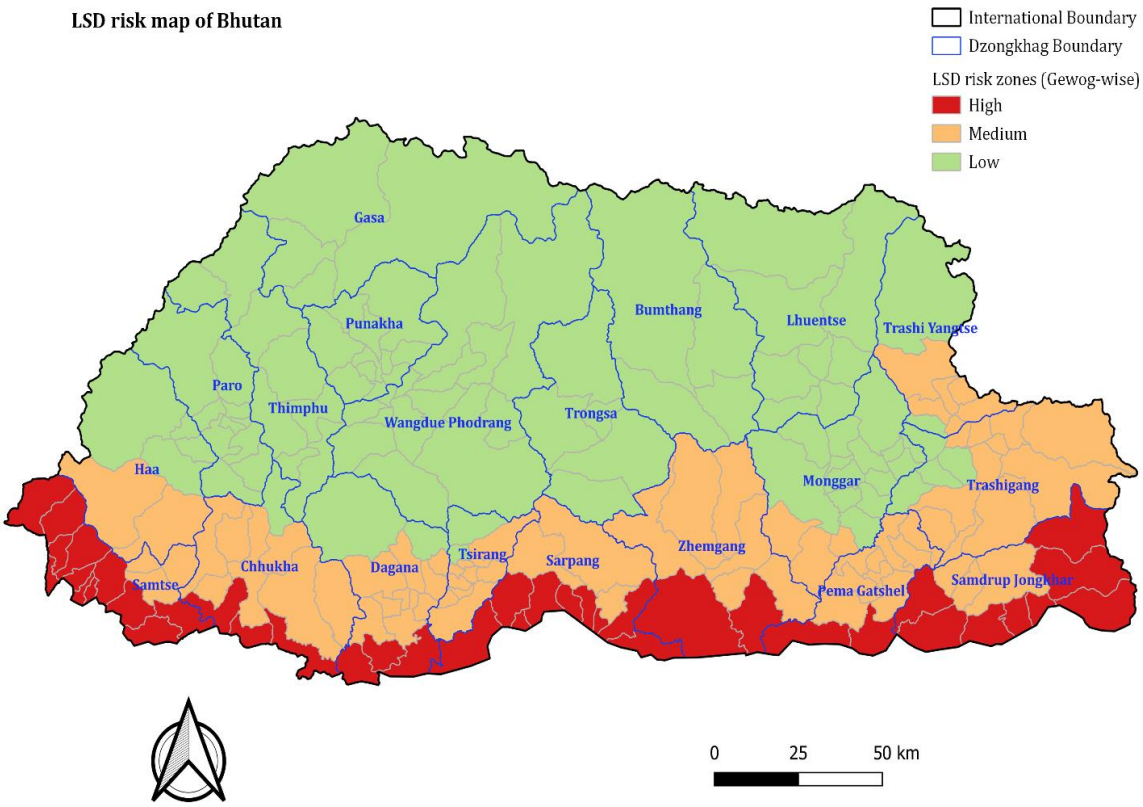


Figure 14: LSD risk map of Bhutan

4. VETERINARY SERVICE STRUCTURE

The animal health services in the country are delivered to farming communities through a network of Extension Centres: Veterinary hospitals and RNR-ECs or LECs. Technical and laboratory supports are provided by National Centre for Animal Health (NCAH), Regional Livestock Development Centres (RLDCs), Satellite Veterinary Laboratories (SVLs), and Dzongkhag Veterinary Hospitals (DVHs) at their respective levels.

4.1. Department of Livestock

The Animal Health Division (AHD) with the Department of Livestock shall oversee policy formulation related to the National LSD prevention and control plan in the country. The specific roles include the following:

- Mobilize resources including the fund for LSD prevention and control programme in the country.
- Liaise with different international organizations/agencies/stakeholders (e.g., FAO, OIE, SAARC) for facilitating the better implementation of the LSD prevention and control programme.
- Collaborate with BAFRA to enable better enforcement of the Livestock Act of Bhutan, and Livestock Rules and Regulations.
- Collaborate with relevant national agencies for ensuring mobilization of support required for LSD prevention and control activities.
- Coordinate border harmonization meetings with the Indian counterparts at the state and central levels.

4.1.1. National Level

The National Centre for Animal Health shall function as the national focal agency for the overall planning, coordination, and implementation of the National LSD prevention and control programme in the country. The responsibilities of the national focal agency are to:

- Coordinate the overall implementation of the LSD prevention and control programme in the country.
- Mobilize necessary resources at the national level.
- Support activation of the rapid response team (RRT) in the event of LSD outbreak(s).
- Liaise with different stakeholders/agencies for facilitating better implementation and ensuring the success of the prevention and control programme.
- Coordinate conduct of epidemiological research on LSD in collaboration with national and international diagnostic and research institutions.
- Production of educational materials and make available for wider circulation for the advocacy campaigns.

- Ensure maintenance of the database on LSD prevention and control programme, analysis and dissemination of information/progress to the Department/ Ministry/ other relevant stakeholders.
- Conduct LSD coordination workshops at the national level to review and realign the prevention and control programme.
- Capacity building for RRT members.
- Monitor and evaluate the prevention and control programmes implemented by the field units.
- Declaration of risk zones/compartments for LSD.
- Maintenance of the status of these zones/ compartments by regular surveillance and monitoring.
- Standardization of protocol for diagnosis of LSD and ensuring uniformity across diagnostic laboratories in the country.

4.1.2. Regional Level

The Regional Livestock Development Centres (RLDCs) and Thromde Veterinary Hospital and Satellite Laboratories (TVH&SLs) shall function as the regional focal agency for LSD prevention and control programme at respective regions and bordering areas. The main roles of the regional focal agency are to:

- Coordinate the overall implementation of the LSD prevention and control programme at the regional level.
- Coordinate the activation of the rapid response team (RRT) in the event of an LSD outbreak.
- Provide support and coordinate logistics arrangement at the regional level and border locations.
- Coordinate the cross-border surveillance for LSD in their respective areas.
- Liaise with BAFRA at the regional level for facilitating better enforcement of the Livestock Act of Bhutan, and Livestock Rules and Regulations.
- Monitoring and evaluation of the LSD prevention and control programmes in their respective regions.
- Ensure prompt reporting of the outbreak and updating/validation of disease status in the disease reporting database system.
- Ensure maintenance of the database on the LSD prevention and control programme and submit the progress report to the NCAH.

4.1.3. Dzongkhag Level

At the Dzongkhag level, the Dzongkhag Veterinary Hospital (DVH) shall function as the focal agency for the implementation of the LSD prevention and control programme. The Dzongkhag focal agency should carry out the following tasks:

- Implement the LSD prevention and control programme at the Dzongkhag level.

- Arrange logistics at the Dzongkhag level and assist the Gewog staff with necessary logistics.
- Liaise with the BAFRA at the Dzongkhag level for facilitating better enforcement of the Livestock Act of Bhutan, and Livestock Rules and Regulations.
- Support the activation of RRT in the event of an LSD outbreak.
- Ensure prompt reporting of the outbreak to the RLDC and NCAH and update the disease status in the animal disease information system.
- Ensure maintenance of a database on LSD prevention and control programme at Dzongkhag Level.

4.1.4. Gewog Level

The Livestock Extension Centre/RNR Extension Centres in the Gewog should be the focal agency for the implementation of the LSD prevention and control programme in that Gewog. The main roles of the Gewog focal agency are as follows.

- Implement the LSD prevention and control programme in the field as per the NLSDPCP.
- Ensure prompt reporting of the outbreak to DVH, RLDC and NCAH.
- Conduct regular disease awareness campaign for the farmers and other clients.
- Implement provisional emergency control measures in the locality in the event of an outbreak.
- Liaise with the BAFRA for facilitating better enforcement of the Livestock Act of Bhutan, and Livestock Rules and Regulations.
- Liaise with the Gewog administration, gups, other local leaders and farmers for facilitating the proper implementation of the prevention and control programme in the field.
- Support RRT for the rapid containment of LSD outbreak(s) in the Gewog.

4.2. Bhutan Agriculture & Food Regulatory Authority

Bhutan Agriculture and Food Regulatory Authority (BAFRA) as a Regulatory Authority under the Ministry of Agriculture and Forests (MoAF) is mandated to enforce and implement the Livestock Act of Bhutan and Livestock Rules and Regulations.

- Enforcement of Livestock Act of Bhutan, 2001 and Livestock Rules and Regulations, 2017.
- Enforcement of movement ban on livestock and livestock products in and out of the LSD outbreak areas.
- Strict quarantining of imported animals at quarantine stations.
- Quarantining of infected animals in the affected areas.
- Monitor the livestock movements from one dzongkhag to others.
- Inspection and certification of suspected livestock products.
- Carry out bio-security measures during the outbreaks (segregation, disposal, cleaning, and disinfection).

- Support activation of the rapid response team (RRT) in the event of an LSD outbreak.
- Border vigilance for the illegal movement of livestock and livestock products.

4.3. Other stakeholders

4.3.1. Department of Forests and Park Services

With the traditional system of livestock farming predominantly practised in most parts of the country where animals are let free in the forest for grazing, interaction with wild ruminants is inevitable. The disease transmission at the domestic-wild life interface cannot be ruled out. Therefore, the collaboration between livestock and forestry sectors is important for disease surveillance, sharing of disease outbreak information, and implementation of prevention and control measures.

4.3.2. Dzongkhag Administration

The Dzongkhag, Dungkhag, and Gewog administration supports are important for coordinating LSD prevention and control activities including rapid containment of LSD outbreak in their jurisdictions.

4.3.3. Royal Bhutan Police

The Royal Bhutan Police support shall be sought if necessary, during the implementation of control measures during the LSD outbreak in the field.

4.3.4. Ministry of Finance

The Ministry of Finance (MoF) must provide adequate funds for the implementation of NLSDPCP in the country. The additional fund support should be sought from MoF if the existing budget is not sufficient during the implementation of emergency response activities.

4.3.5. International collaboration

It is important to build linkages with international organizations such as the United Nation's Food and Agriculture Organization (FAO), the World Organisation for Animal Health (OIE) and others for seeking fund and technical supports, human resource development, and referring of samples for laboratory diagnostic services. The national laboratory will also seek support from the OIE LSD reference laboratories.

5. PREVENTION AND CONTROL STRATEGY

5.1. Regulatory framework

5.1.1. International legislation

The OIE standards mentioned in various sections and chapters of the OIE Terrestrial Animal Health Code (TAHC) 2019 guides the relevant sections of this document. The TAHC 2019 guides animal disease diagnosis, surveillance and notification; risk analysis; veterinary services, disease prevention and control; trade measures, import/export procedures and veterinary certification; and animal welfare. Chapter 11.9 of the document titled "Infection with lumpy skin disease virus" guides the importation of susceptible animals and their products and LSD surveillance.

5.1.2. National Legislation

The following documents are in force concerning reporting, prevention, and control of notifiable and emerging/exotic diseases of national importance, transport of animals, quarantine, import of animal, welfare, and biosecurity.

- Livestock Act of Bhutan, 2001
- Livestock Rules and Regulations of Bhutan, 2017
- Bhutan Health Code for Import of Animals, 2018
- In-Country Livestock Biosecurity Guidelines, 2015
- Animal Quarantine Station Operation Manual
- Guidelines on animal tshethar practices 2018
- Bhutan Biosafety Act 2015
- Bio-safety Rules and Regulations, 2018

Although LSD is not listed as a notifiable disease in Bhutan, owing to its paramount socio-economic impact on farmers and the government through direct and indirect losses, the disease, on mere suspicion, must be notified to the nearest animal health centre and further. However, the Department of Livestock is in the process of incorporating LSD into the Notifiable Disease list. During the interim period until when the incorporation is officially endorsed, the animal health authority shall report any even suspected LSD. Chapter IV of the Livestock Rules and Regulation 2017 empowers the concerned offices to designate notifiable disease, destroy animals, animal products and feed posing risks, ban on shows and sale of livestock and its products during the outbreaks, quarantine imported animals and their products, and restrict animal movement.

Chapter 10 of the Bhutan Health Code for Import of Animal 2018 clearly states that the incursion of exotic diseases through the import of animals will be dealt with as per OIE Terrestrial Animal Health Code and Aquatic Animal Health Code or based on the findings of the risk assessment conducted by DoL and BAFRA.

5.2. Disease surveillance

5.2.1. General principles of surveillance

The surveillance strategy should be adequate to detect the presence of infection with LSDV even in the absence of clinical signs, given the prevailing epidemiological situation as per Chapter 1.4 (Animal health surveillance) and Chapter 1.5 (Surveillance for arthropod vectors for animal diseases) of the TAHC 2019.

The Veterinary Services should implement programmes to raise awareness among farmers and workers who have day-to-day contact with livestock, as well as veterinary paraprofessionals, veterinarians and diagnosticians, who should report promptly any suspicion of LSD.

As required, Bhutan has in place, a formal and ongoing system for detecting and investigating cases; a procedure for the rapid collection and transport of samples from suspected cases to a laboratory for diagnosis; and a system for recording, managing and analysing diagnostic and surveillance data.

5.2.2. Clinical surveillance

Clinical surveillance is essential for detecting cases of infection with LSDV and requires the physical examination of susceptible animals. Surveillance based on clinical inspection provides a high level of confidence in the detection of disease if a sufficient number of clinically susceptible animals is examined regularly at an appropriate frequency, and investigations are recorded and quantified. Clinical examination and laboratory testing should be pre-planned and applied using appropriate types of samples to clarify the status of suspected cases. For detailed clinical signs and lesions, refer to the clinical diagnosis section (Section 2.3.1) of this document.

5.2.3. Virological and serological surveillance

An active programme of surveillance of susceptible populations to detect evidence of infection with LSDV is useful to establish the status of the disease in the country or a zone. Serological and molecular testing of bovines and water buffaloes may be used to detect the presence of antibody due to exposure and agent, respectively. The study population used for a serological survey due to infection should be representative of the population at risk in the country or zone and should be restricted to susceptible unvaccinated animals. Identification of vaccinated animals may minimise interference with serological surveillance and assist with recovery of free status.

5.2.4. Surveillance in high-risk areas

Disease-specific enhanced surveillance in a free zone should be carried out over an appropriate distance from the border with an infected country or zone, based upon

geography, climate, history of infection and other relevant factors. The surveillance should be carried out over a distance of at least 20 kilometres from the border, but a lesser distance could be acceptable if there are relevant ecological or geographical features likely to interrupt the transmission of LSDV. Surveillance may be conducted in areas where there is a high prevalence of arthropod vectors such as flies, mosquitoes and ticks.

5.3. Chain of reporting for LSD

Like any notifiable animal disease reporting system in the country, a suspected case of LSD must be reported by farmers/ animal owners to the nearest animal health extension centre.

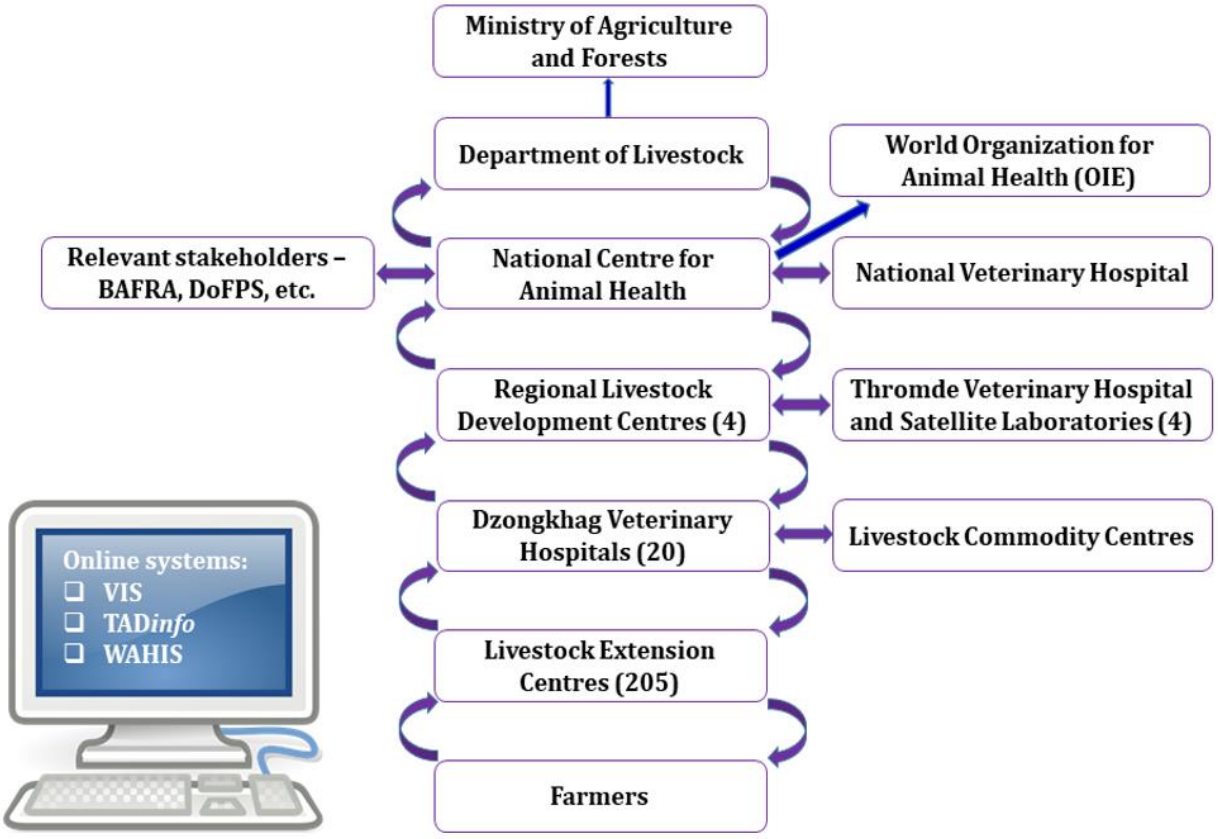


Figure 15: LSD notification flow-chain

The centre should further report to the district or regional office, whichever relevant. Finally, the syndromic surveillance report, in the form of a flash report (Annexure 3, Form 1), should be submitted to the NCAH, where further activities shall be carried out: data compilation, validation, analysis and communicating to relevant stakeholders. During the process, corresponding level (district/regional) BAFRA and other relevant stakeholders should also

be reported. The detailed chain of reporting regarding LSD suspected or confirmed cases is given in the Figure 15.

5.4. Diagnostic capability

The LSD Prevention and Control Programme should be supported by diagnostic facilities with adequate capability. Samples for diagnosis should be collected and stored following the SOP for sample collection for LSD, developed by the National Veterinary Laboratory, NCAH, aligned with the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019 (Annexure 2, SOP 4). Diagnostic tests should follow the specific requirements in Chapter 1.1.6 on principles and methods of validation of diagnostic assays for infectious diseases and the disease specific recommendations in the Terrestrial Manual. Diagnostic facilities should be under a quality assurance scheme coordinated by the National Veterinary Laboratory, NCAH, Serbithang. The NCAH should establish communication with OIE Reference Laboratories for LSD and conduct proficiency test for LSD diagnosis and characterization. The Regional and Satellite Veterinary Laboratories should ensure that diagnostic results are communicated to the Veterinary Authority as appropriate to the situation. The NCAH should submit samples to OIE reference laboratories for confirmation of findings and detailed analysis. The Department of Livestock should strengthen the higher level of diagnostic capacity for LSD at the national and regional laboratories through the development of HR capacity on the LSD sampling and diagnosis. The field laboratories may use rapid diagnostic techniques.

5.5. Traceability

An effective traceability system is imperative for the identification of affected animals, herds, or flocks during the event of an LSD outbreak. It significantly improves the effectiveness of activities such as the management of disease outbreaks, early warning response and notification system, vaccination programmes, surveillance, animal husbandry, zoning or compartmentalization, and movement of animal and their products at all levels.

Currently, BAFRA has the Bhutan Bio-security System (BBSS) which is a web-based portal system to manage the import of animals and the movement of their products within the country as a part of the traceability system. The system captures the information on animal population parameters such as species, breeds, numbers, distribution, types of production, animal movement patterns, trade in animals and animal products. The BBSS should generate up-to-date and timely information on the movement of animals and their products regarding import and movement within the country leading to efficient movement control and collation of data. Such data should be shared among other stakeholders such as the National Centre for Animal Health under the Department of Livestock.

5.6. Biosecurity measures

Provisions under Section 9 of the Livestock Act of Bhutan 2001 require the implementation of robust biosecurity measures to prevent and control the spread of livestock diseases. Farm biosecurity is a strategic and integrated approach to analysing and managing relevant risks to human health, animal health and associated risks to the environment. A robust biosecurity measure plays an integral part in disease prevention and control measures both during peace and outbreaks time, and the measures encompass a continuum along the disease risk pathway from a disease source outside Bhutan's border, through the border, and to the farm (post-border) where disease outbreaks may occur and have a significant impact.

If possible, live cattle or water buffaloes and their products should be imported from countries or zones free of LSD. However, if they are imported from countries or zones which has recorded LSD outbreaks recently or in the past, strict biosecurity measures should be implemented.

5.6.1. Pre-border biosecurity measures

Since LSD is a transboundary disease, any import of LSD susceptible animals and their products shall be accompanied by prior import permits approved by BAFRA. The import permits shall clearly define import conditions as per the Bhutan Health Code for Import of Animal 2018 or after carrying out science-based risk analysis following OIE standards. The disease-free certificate and vaccination record against certain notifiable diseases issued by the veterinarians from their jurisdiction shall be declared by the concerned individuals and validated before shipping the animals to border areas for quarantine.

5.6.2. Biosecurity measures at the border

As empowered by the Livestock Rules and Regulation 2017, BAFRA has designated animal quarantine stations at borders and the international airport with a facility for animal quarantine and inspection of animal for any suspected notifiable diseases to prevent the entry of diseases and other hazards of animals into the country. All animals imported into the country must undergo 15 days mandatory quarantine period. During the quarantine period, the samples are collected and tested for screening of exotic, notifiable, and zoonotic diseases.

5.6.2.1. Import of live animals

For the importation of cattle and water buffaloes, an official veterinary certificate must be attested certifying that the animals:

- showed no clinical sign of LSD on the day of shipment.
- were kept since birth, or for the past 60 days before shipment, in an epidemiological unit where no case of LSD occurred during that period.

- were vaccinated against LSD according to manufacturer's instructions between 60 days and one year before shipment.
- were demonstrated to have antibodies at least 30 days after vaccination.
- were kept in a quarantine station for the 28 days before shipment during which time they were subjected to an agent identification test with negative results.

5.6.2.2. Import of livestock products

Regulation of the movement of animal products requires a coordinated approach. It should require the presentation of a certificate from relevant veterinary authority:

- these products were derived from animals that have been kept in a country or zone free from LSD since birth or for at least the past 28 days; or
- these products were processed to ensure the destruction of the LSDV and the necessary precautions were taken after processing to avoid contact of the commodities with any potential source of LSDV.

The inspector at the border shall verify the necessary documents required for the import conditions in the import permit which only on fulfilment shall be allowed inside the country for sale or consumption.

5.6.2.3. Monitoring of illegal movement of animals and their products

Bhutan shares a porous border with India in the south and China in the north. The uncontrolled illegal movement of animals across international borders especially in the south presents a serious threat of incursion of LSDV into Bhutan. In such a situation, an external coordination mechanism between the animal health authorities, livestock importers, regulatory authority, and law enforcement agencies (revenue and customs, immigration, and police) should be developed from the national to local level to curb such issues. This should include an education campaign on the direct and indirect impact of LSD outbreaks through such illegal practices on the target population. The cooperation leading to simple, practical quarantine and disease surveillance procedures should be encouraged.

On the other hand, BAFRA should strengthen the risk-based border vigilance surveillance to intercept the illegal movement of animals and their products supported by the legislative framework. The quarantine facilities at the border should have the capacity to intercept illegally imported animals and products. The illegal import of live animals anticipated should be quarantined and any confiscated risk materials should be disposed of safely by deep burial or incineration.

5.6.3. Post-border biosecurity measures

Biosecurity measures at post-border are important during the peace period and outbreak time. During the peace period, the regulatory authority shall implement stringent biosecurity measures in the farms and movement of animals and products within the country. BAFRA has developed an online Processing System of In-Country Movement Permit for Live Animals

and a biosecurity checklist for commercial dairy farms in the country. The document should continue to reflect a mutual agreement on the best practices for livestock biosecurity so that the 'biosecurity message' is clear and regularly reinforced to all livestock farmers. As mandated by the Livestock Rules and Regulation 2017, the regulatory authority shall carry out inspection and monitoring of on-farm biosecurity in livestock farms periodically for continuous improvement during the peace period.

In the country, there is a risk for LSDV incursion through Tshethar practices. Accordingly, to mitigate such incidences, the guideline on animal Tshethar Practices 2018 was developed, by which import of animals for Tshethar practices is prohibited. Furthermore, the biosecurity measures in the animal shelter in Tshethar animals shall be regulated as per the guidelines.

5.6.4. Biosecurity measures during the outbreak

5.6.4.1. Ban on the movement of livestock and livestock products

Ban on the movement of all susceptible animals into and out of the affected area and their products (such as milk, meat, butter, cheese, hides, etc.) both into and out of the affected areas should be strictly implemented to prevent the spread of the infection to other places. BAFRA should mobilize their staff to attend the check posts and entry points and to strictly control the movement of livestock and livestock products in and out of the outbreak area.

5.6.4.2. Zoo-sanitary measures

The carcass of an animal that died due to LSD should be properly disposed of to avoid the spread of the virus through contamination (Annexure 2, SOP 2). All the staff and motor vehicles exiting the infected areas should be properly disinfected as it exits the affected areas.

5.7. Vector control

Efficient insect control on cattle or in the holding may reduce the rate of mechanical transmission, but cannot prevent it, particularly when cattle are free-roaming or kept in fenced pastures. The application of spot-on repellents can work for the protection of cattle from insects for a short time.

When insecticides are used, withdrawal times for milk and meat need to be considered.

Limiting vector breeding sites, such as standing water sources, slurry, and manure, and improving drainage in the holding are sustainable, affordable, and environmentally friendly ways to reduce the numbers of vectors on and around cattle.

5.8. Regional and international collaboration

5.8.1. Border harmonization

Coordination meetings with the state veterinary departments of the adjoining Indian states (Assam, West Bengal, and Arunachal Pradesh) are needed to help for common understanding and the development of collaborative efforts for disease control in both countries. The Department of Livestock under the Ministry of Agriculture and Forests should interact with the Department of Animal Husbandry and Dairying, Government of India for bringing about understanding between the two countries and to revitalize the border harmonization meetings (BHM) with the Indian counterparts. The Department of Livestock shall coordinate BHM with support from relevant agencies.

5.8.2. Regional and international effort

LSD is a transboundary animal disease and requires a regional approach in its prevention and control. Since neighbouring countries often have similar socio-economic, epidemiological and livestock production systems, there are similar risks for livestock diseases. Bhutan in this situation shall consider sharing resources in the LSD prevention and control programme. Considerable mutual benefits can be derived from neighbouring countries which can be done through informal networking or more formally, through existing regional organizations: OIE, the Animal Production and Health Commission for Asia and the Pacific (APHCA) and the Association of Southeast Asian Nations (ASEAN) in Asia. A regional approach with a well-coordinated programme is far more likely to succeed in the prevention and control of LSD in Bhutan. The potential areas for collaboration include:

- Joint risk assessments leading to the harmonization of import quarantine policies and other disease prevention strategies in the region.
- Joint coordination of disease surveillance, vaccination, and others in the region.
- Information sharing on disease outbreaks.
- Resource sharing: diagnostics
- HR capacity development training programmes.

5.9. Advocacy and awareness/social participation

Public awareness and education about the disease and its impact is an integral part of the LSD prevention and control plan. The livestock farmers and animal traders that will be affected by LSD and its control actions should be targeted. The most appropriate means of spreading the message across to specific communities should be used, such as radio broadcasts and village meetings. The latter are particularly suitable since they give people the opportunity to ask questions and material (such as pamphlets and posters) can be disseminated that will reinforce the information given. The campaigns should inform people

of the nature of the disease and its negative impacts, reporting suspected cases, and the benefits. Public awareness materials that are targeted specifically at all stakeholders should be prepared.

6. PREPAREDNESS AND EARLY WARNING

6.1. Simulation Exercises

Simulation exercises are extremely useful for testing and refining the National LSD Prevention and Control Plan which can be taken up as either table-top or field or combining both approaches based on the availability of resources. A simulation exercise is done to test the latest version of the LSD prevention and control plan in the field by simulating an outbreak with field veterinarians and veterinary paraprofessionals and other stakeholders. This is also done to test their skills in LSD control. Realistic disease outbreak scenarios shall be developed for the exercises, using real data where possible (e.g., for livestock locations, populations) preferably in the disease-endemic areas. Simulation exercises can be conducted to test one or multiple components of the disease prevention and control plan. After each simulation exercise, an assessment or review of the results shall be conducted by the identified observers. Such a review will identify areas where the plans must be modified and determine the need for further training. A full-scale disease outbreak simulation exercise shall be conducted after individual components of the disease control response have been tested and proved.

6.2. Early Warning System

Early warning of the outbreaks and the capacity for prediction of spread to new areas is an essential prerequisite for the effective containment of animal disease epidemics. Weaknesses of disease surveillance systems and the inability to control their source have contributed to the spread of diseases across the borders. Early Warning and Response is based on the concept that dealing with a disease epidemic in its early stages is easier and more economical than having to deal with it once it is widespread.

The National Centre for Animal Health shall relay the LSD outbreaks information to field colleagues and relevant stakeholders, thus, reminding them to prepare for immediate response. Currently, real-time update of disease outbreaks situation is shared through the NCAH webpage, and near real-time information sharing through fortnightly e-bulletin on animal disease information of Bhutan. The information can also be accessed through an online animal disease information system. The NCAH shall explore other methods of rapid information sharing mechanism like the development of user-friendly mobile applications to enhance disease outbreak reporting system.

During the outbreaks of LSD in the region and neighbouring countries, surveillance activities shall be heightened at high-risk border areas and implemented through the activation of Veterinary Vigilance Team comprising veterinarians and veterinary paraprofessionals under the DoL and Border Vigilance Team comprising officials under BAFRA.

7. LSD OUTBREAK RESPONSE STRATEGY

7.1. LSD Case Definitions

Case definitions for Lumpy skin disease are as described in the following table.

Table 3: Case definitions for LSD

Case	Definition
Suspect case	Cattle and buffalo showing clinical signs suggestive of LSD: intradermal nodules on the skin and mucous membranes which later ulcerate. Enlarged lymph nodes, laboured breathing, fever, and oedema of the forelimbs may also occur.
Probable case	A suspected case with the history of the introduction of new animals, epidemiologically linked to LSD outbreaks and showing clinical signs suggestive of LSD.
Confirmed case	Antigen-specific to LSD, excluding vaccine strains, or antibodies to LSDV antigens which are not the consequence of vaccination, has been identified from samples of a suspected, probable, or asymptomatic case.

7.2. Early Detection

On mere suspicion of LSD cases, the livestock owners/farmers should immediately report to the nearest livestock or animal health centre in the Gewog. The Gewog Livestock Office should immediately investigate all suspected clinical cases of LSD and declare a "suspected LSD outbreak" if the affected animal shows typical signs suggestive of LSD (see suspect case definition in Table 3). Following this, the concerned Gewog livestock staff should immediately report to the DVH, RLDC, and the NCAH using the standard Flash Report Form (Form 1) or by the fastest means of a communication channel.

7.3. Declaration of Provisional Infected Zone

During the preliminary investigation of LSD suspected cases, Gewog authority should immediately designate the suspected infection zone (farm or a village) and the surrounding area (based on risk assessment) as a Provisional Infected Zone. The geographical limits of the Provisional Infected Zone should be determined after due consideration of the epidemiological risk and natural geographical settings. This provisional zone shall be declared through official order by the concerned Gewog administration office based on the recommendation of the disease outbreak investigating officer or team.

The following control measures should be implemented in the Provisional Infected Zone to prevent the spread of the suspected disease.

- Immediate segregation of affected animals including separate management (feeding, watering, milking, etc.)
- Symptomatic treatment of the affected animals
- Disinfection/Decontamination of the contaminated premises (SOP 3).
- Provisional ban on the movement of susceptible animals and their products from the suspected infected premises
- Vector control measures
- Awareness and education for the livestock owners in the affected village on-farm biosecurity measures

Surrounding the provisional infection zone, depending on the disease epidemiology and geographical settings, a provisional protection zone shall be delineated.

7.4. Outbreak Investigation

An outbreak investigation, also called the epidemiological investigation or epidemiological enquiry, is a specialized activity carried out by the veterinary services. In essence, it merges standardized data gathering procedures and scientific method with the aim of better understanding the behaviour and risk factors of a disease, in this case, LSD. The main objective of outbreak investigation is to find answers to the following 3 questions:

a) Where did the infection come from?
b) How long has the infection been present?
c) Where could the infection have spread to?

It is important to maintain records of outbreak investigations including those in which the disease was not confirmed, as this demonstrates the effectiveness of the surveillance system. The DVH/RLDC should send a disease outbreak investigation team to investigate the suspected case immediately (SOP 1). Upon the confirmed cases of LSD from NVL, NCAH, the investigation team should undertake a comprehensive epidemiologic assessment in the field including appropriate sample collections and testing. to confirm the case as well as to collect appropriate samples. The clinical diagnosis should be further confirmed with laboratory diagnosis at the NCAH. Samples should also be referred to OIE designated reference laboratories for the characterization of the LSD virus. The disease outbreak investigation team should also update the detail of the outbreak through an online database system followed by a weekly update of the disease outbreak situation. The BAFRA and other relevant offices in the Dzongkhag should also be notified.

The outbreak investigation should be done as per the flowchart process shown in Figure 16.

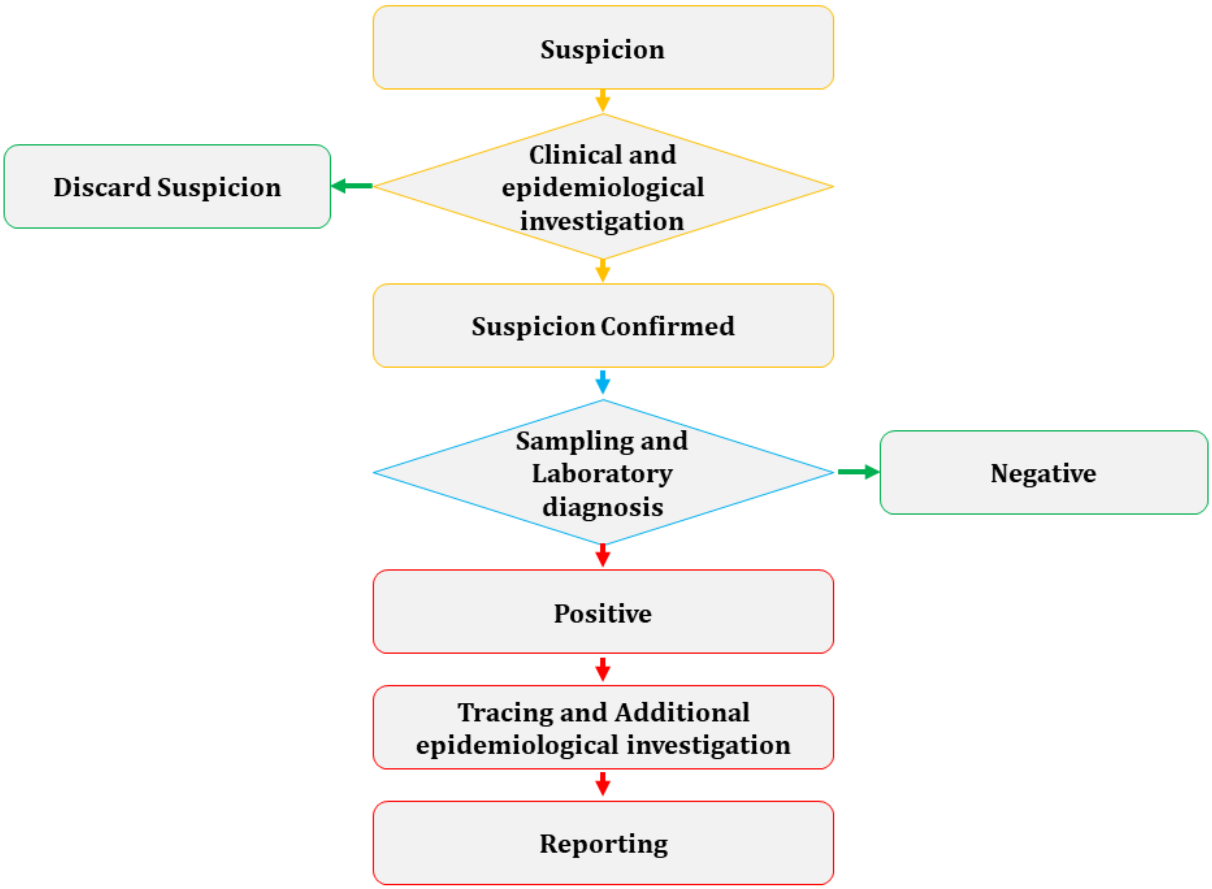


Figure 16: Flowchart of the outbreak investigation process

The following steps should be followed while undertaking an outbreak investigation:

- Describe the outbreak in terms of animal, place and time.
- Look at the risk factors and establish a hypothesis.
- Take steps to confirm the disease – clinical history, clinical examination, sample collection and confirmation.
- Define and count the cases – based on case definitions.
- Define the population at risk.
- Implement control measures.
- Document the investigation – systematic records of investigations conducted, including photographs if possible.

The following data should also be included in an outbreak investigation:

- number of animals in the herd, number of suspected animals, estimated age of lesion(s).
- origin, age, sex, breed, production type and vaccination status of suspected animals.
- contacts with other herds and use of communal grazing – contacts with wild ruminants.

- cattle movement records – new animals recently introduced into a herd and their origin; animals that have left the herd and their destination.
- movements of animal care staff and other visitors.
- recent veterinary treatments and cattle health records.
- artificial inseminator visits and use of a breeding bull.
- milk collection vehicle.
- animal trader/slaughterhouse transport vehicle visits: any farms visited before and after.
- potential vector activity, presence of vector breeding sites such as lakes, rivers.
- road network, other geographic and climatic data.
- a survey of the premises should be made, and potential vector breeding sites removed.

7.5. Declaration of the Infected and Protection zones and LSD outbreak

After the laboratory confirmation of LSD, the area where the disease has occurred within a radius as decided by the disease outbreak investigation team should be immediately delineated and declared as the Infected Zone. The geographical limits of the infected zone should be determined after due consideration of the epidemiological risk and natural geographical settings. The disease outbreak investigation team should also declare a protection zone outside the radius of the infected zone, where active surveillance shall be conducted by the clean team. Based on the recommendation of the disease outbreak investigation team, the Dzongkhag administration should issue the disease outbreak declaration order with information to the Gewog Administration, DVH, BAFRA, RLDC, NCAH, and the DoL.

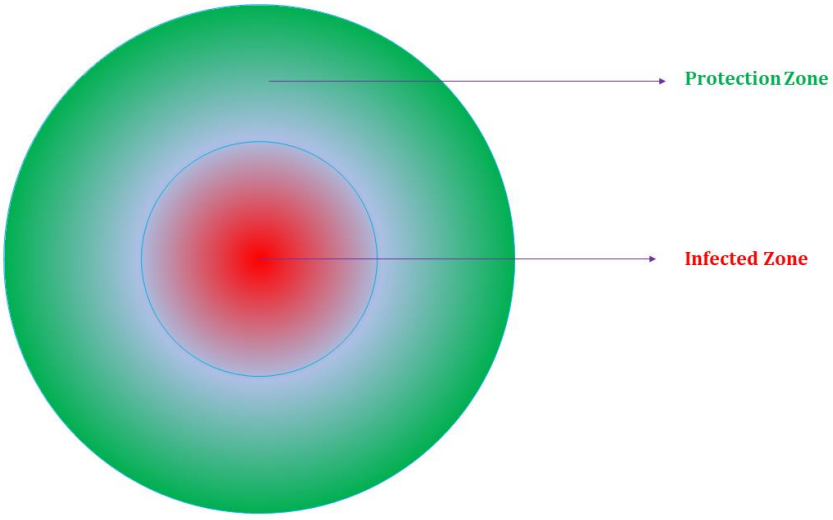


Figure 17: Declaration of infected and protection zone

All the provisional control measures should be continued with reinforcement of the efforts in the designated locations once the disease outbreak is officially declared. If the disease suspected is not LSD, all the provisional control measures that are being implemented should be immediately discontinued and the alternate measures specific to that disease should be undertaken in alignment with the available disease-specific plan or recommended measures.

7.6. Activation of the Rapid Response Team

Once the LSD outbreak is confirmed by the disease outbreak investigation team and substantiated by laboratory confirmation, Rapid Response Team (RRT) should be activated immediately to contain the disease without allowing it to spread to other places. The RRT should mainly constitute of three main technical groups to effectively implement disease control measures:

i. Disease outbreak investigation team
ii. Quarantine and movement control team
iii. Surveillance team

If required, depending on the scale of outbreak and response measures implemented, a logistics team may be deployed.

The Department of Livestock should be responsible for disease outbreak investigation, treatment of sick animals, surveillance, and logistic supply. BAFRA should be responsible for quarantine and movement control of susceptible livestock and livestock products from the infected zone. The roles of various teams under the RRT are described in Annexure 1.

Following activities should be implemented by the RRT to effectively control the LSD outbreak:

- **Isolation and treatment of affected animals**

All the affected animals should be isolated and provided symptomatic treatment to prevent mortality and to enable faster recovery. A separate team (preferably the livestock staffs who already had contact with the sick animals) should be involved in the treatment of the sick animals as well as for implementing the zoo sanitary measures in the affected areas.

There is no effective treatment available for LSD. To lower the fever and keep animals eating, anti-inflammatory painkillers may be used. In some cases, antibiotics need to be used to treat secondary bacterial infections.

- **Farm biosecurity**

LSD virus is very stable and survives well in extremely cold and dry environments. Infected animals shed scabs from skin lesions to the environment. Inside of these scabs, the virus may remain infectious for several months.

Thorough cleaning and disinfection using effective disinfectants, as listed in Table 2, should be performed on the affected farm, trucks, equipment, personnel's clothes, premises, and potentially contaminated environment.

Although LSDV is sensitive to most disinfectants and detergents, mechanical removal of surface material such as dirt, manure, hay, and straw are required before disinfection. The disinfectant used should be able to penetrate the organic material that the infectious virus may be surrounded by in the environment.

All the staff exiting the infected areas should strictly disinfect themselves while leaving the affected areas (SOP 3).

- **Ban on the movement of livestock and livestock products**

Ban on the movement of all susceptible animals including their products (such as milk, meat, butter, cheese, hides, etc.) both into and out of the affected areas should be strictly implemented to prevent the spread of the infection to other places. BAFRA is responsible to regulate the movement of livestock and livestock products in and out of the outbreak area.

Time and area for imposing restrictions shall be decided based on the risk assessment conducted by the Disease Outbreak Investigation Team.

- **Surveillance and weekly follow-up**

The Disease Outbreak Investigation Team should conduct active surveillance in the infected zone, whereas the clean Surveillance Team shall conduct active surveillance in the protection zone for early detection of LSD case(s). An update on the disease outbreak situation should be done as a follow-up report every week through the existing disease outbreak reporting channel.

When LSD outbreak(s) is detected and ongoing in the country, surveillance activity should not be restricted only to the infection and protection zones. Disease surveillance activities should be heightened in all the Dzongkhags and Gewogs in the country.

- **Logistic support**

To implement measures for successful containment of LSD outbreak by RRTs, adequate logistic support should be arranged by the concerned RLDC, in consultation with the Department. Logistic supports are needed in the form of manpower, mobility, and funds to execute the implementation of various LSD prevention and control measures.

- **Lifting of disease control zones and movement control bans**

The infection and protection zones demarcated, and the movement control bans imposed earlier should be lifted 28 days after the last detected case; however, the duration is subjected to change depending on the risk assessment conducted. Once the disease control zones are lifted, the movement of livestock and the sale of livestock products can resume. However, routine surveillance and prevention activities must be continued.

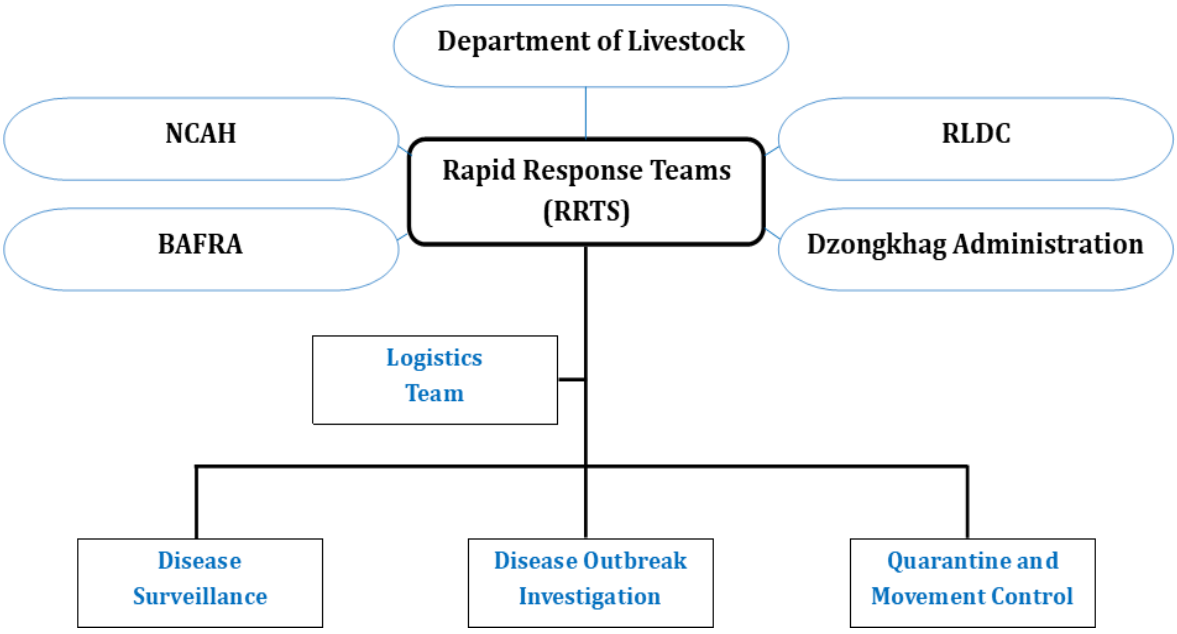


Figure 18: Rapid response teams during LSD outbreak(s)

8. LSD SURVEILLANCE SYSTEM

Surveillance activities include active and passive clinical surveillance and laboratory testing of blood samples, nasal swabs, or skin biopsies collected from suspected cases.
Surveillance is needed to understand the health status of the animals in the country so that problems can be identified, and actions are taken. For an appropriate surveillance activity to be performed, we should have a clear objective and understanding of the need. There are large numbers of reasons why veterinary authorities undertake surveillance activities and can be summarized into four general purposes:

- Demonstrating freedom from disease
- Early detection of disease
- Measuring the level of disease
- Finding cases of the disease

Surveillance is a key element of the national LSD prevention and control plan.

8.1. Clinical surveillance

Continuous passive surveillance will be done along with other notifiable diseases. Any cases with skin nodular lesions in cattle and buffaloes must be reported and investigated by field veterinary paraprofessionals. Any clinical cases suspected will be followed up by a collection of appropriate samples and referral to designated laboratories for confirmation.
Active surveillance (structured non-random surveillance, including targeted or risk-based surveillance) shall be conducted in the southern and nearby high-risk districts. Syndromic surveillance, participatory disease search, a sentinel system, and abattoir surveillance should also be conducted. Each livestock centres in the high-risk areas should collect information on LSD status in animals from their respective areas (village and farms). The reporting should be done from Gewog to the Dzongkhag office. The Dzongkhag Livestock Sector (DVH) should then relay the information to the RLDC and NCAH. In events of suspected LSD cases, Form 1 should be used to flash the cases from the field to DVH, RLDC, and the NCAH. Simultaneously, the case will be updated in the online animal disease information system by the focal persons. The NCAH shall maintain national data, perform analysis, and communicate to all the stakeholders in the country and beyond.

8.2. Surveillance during the outbreak

Once the LSD outbreak is confirmed (clinical and laboratory-based) by NVL, NCAH, continuous surveillance should be carried out in the infected and protection zone by RRTs. To keep relevant stakeholders updated, the disease status shall be submitted every week to the RLDC and NCAH and updated simultaneously into the available online information

system. When the outbreak is effectively contained, the imposed ban on animals and their products shall be lifted 28-day post reporting of the last case.

8.3. Laboratory surveillance

8.3.1. Sero-surveillance

As there is no DIVA (Differentiation of infected from vaccinated animals) vaccine against LSD, serological surveillance is of no use in areas where the cattle and buffalo population are imported from vaccinated areas.
In context to Bhutan, since vaccination against LSD has not been initiated, the presence of seropositive animals can be considered as an indication of recent outbreaks. However, it's important to rule out that the sero-positive animal is not imported from a place where vaccination against LSD being practiced.

8.3.2. Virological surveillance

Virological surveillance will be conducted as a follow-up to clinically suspected cases. During the outbreak period, relevant samples from suspected and infected animals shall be collected and tested using available recommended laboratory diagnostic tests. The molecular analysis will also be conducted to understand the cross-border incursions. LSDV isolates will be sent to an OIE Reference Laboratory for further characterization.

8.4. Surveillance in wildlife

Although the role of wildlife in the epidemiological cycle of LSD and virus circulation is not yet entirely understood, wild ruminants may contribute to the geographic spread of the disease through their migratory movements, which can stretch over long distances. As a part of the LSD surveillance programme, veterinary officials should closely liaise with the forestry officials to investigate the morbidity and mortality of wild ruminants and rule out LSD infection. The forestry officials should also inform the nearest veterinary authority as and when they come across the death of wild ruminants suspected of LSD so that appropriate samples are collected for laboratory examination.

9. OTHER SUPPORT PLANS

9.1. Communication and advocacy

Awareness campaigns need to be intensified and targeted to all cattle sector stakeholders. They should be aware of the risk of the disease, the ways to prevent it, how to recognize it and the need to report it to the veterinary authorities immediately when suspected.
Cattle transport drivers are in a key position to identify infected animals on farms, slaughterhouses, cattle collection holdings and resting stations, and to notify the veterinary authorities of such clinical suspicions as soon as possible.
Awareness campaigns should also be targeted to consumers to regain their trust to use cattle products during and after an outbreak.

Following are the awareness programmes to be implemented during the prevention phase:

- Awareness on LSD and its impact on animals and farmers.
- Awareness through training of stakeholders (farmers, traders, meat vendors, livestock officials) on disease and control measures.
- Awareness on timely reporting of suspected cases.
- Awareness on the National LSD Prevention and Control Plan.

Following are the awareness programmes to be implemented during the outbreak phase:

- Awareness on the regulation of movement of livestock and livestock products.
- Awareness on timely reporting of clinical cases.
- Awareness on safe disposal of animals that died of LSD.
- Awareness of other measures mentioned in the National LSD Prevention and Control Plan.

9.2. Veterinary capacity development

National LSD prevention and control plan require capacity development in diagnostic and surveillance systems. Strengthening the national diagnostic and epidemiological unit that are responsible for LSD surveillance and control programme at the national level is essential. Upgrading diagnostic facilities at the national, regional, and district levels with trained laboratory staff is necessary for the control programme. Veterinarians and veterinary paraprofessionals from DoL and BAFRA must be trained in the diagnosis, sampling and surveillance for LSD.

9.3. Coordination meeting

It is necessary to conduct annual national coordination meetings or workshops with all the stakeholders to review and revise the animal disease prevention and control programme. Besides, the regular coordination meeting should be organised at the regional and Dzongkhag level to smoothly implement the animal disease prevention and control activities in the field to analyse and assess its progress.

9.4. Research works

Although research and studies related to LSD have been conducted and available for reference, the investment in further research, in context to Bhutan, will be invaluable to facilitate the campaign and speed up the course of the programme. The following research should be conducted to support the LSD control and eradication plan:

- Epidemiology of LSD in Bhutan.
- Identify the risk hot spot (zones) for LSD.
- Determine the serotypes of LSDV in animals.
- Conduct risk analysis along the livestock value chain.
- Estimate the benefit-cost of prevention and control programme.
- Estimate the socio-economic impact of LSD on different stakeholders.
- The role of wildlife in the epidemiology of LSD in Bhutan.

9.5. Programme financing

The National LSD Prevention and Control plan will require the development of an annual operational plan including the specification of physical requirements and the estimated costs of the work for every subsequent year. The required budget should be proposed to the Ministry of Finance each year by AHD, NCAH, RLDCs and Dzongkhags based on the roles and activities required to be executed by respective agencies. It will also need a clear specification of the output, key performance indicator, responsibility, and timeline.

The Department of Livestock must propose and secure a contingency fund to implement emergency disease prevention and control measures for preparedness and response to the outbreaks of emerging disease or to support during the outbreaks of re-emerging animal diseases of national importance.

10. MONITORING AND EVALUATION

A robust monitoring and evaluation system are crucial for the smooth delivery of programmes, activities, and related services for the achievement of anticipated outcomes. The success of the National LSD Prevention and Control Plan shall be assessed on an annual basis. Besides, the NCAH shall closely monitor the LSD prevention and control activities in close collaboration with RLDCs and DVHs.

The LSD prevention and control plan must be updated regularly to always fit the purpose. After an LSD outbreak, a thorough evaluation of the efficacy of different control and elimination measures needs to be conducted by involving relevant stakeholders. Update and revision of the plan should be based on the experiences gathered from the previous outbreaks and lessons learnt. In peacetime, full-scale simulation exercises with the involvement of all stakeholders must be performed to test all the components of the plan for future update and improvements.

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ANNEXURE

Annexure 1: Rapid Response Teams (RRT) during LSD Outbreaks

The formation of Rapid Response Teams (RRT) to respond against the outbreak of LSD should be formed with the objectives:

- to conduct a thorough investigation of a disease outbreak(s) for the assessment of the disease/agent as well as to identify the source(s) of the infection.
- to rapidly contain the disease to prevent its spread to other places, which includes certain actions even before the disease/agent is confirmed.
- for risk communication on the disease and its control measures to educate public and field staff.
- to coordinate with the different stakeholders in responding to the disease outbreak within the shortest possible time to reduce the cost of response.

A. Team Members for RRT

- Head of Animal Health Section, RLDC – Team leader.
- Laboratory Technician of RLDC.
- Livestock Health Supervisor, RLDC.
- Dzongkhag Livestock Officer, DLS.
- Veterinary Officer/In-charge, DVH.
- Concerned Regulatory Officials from BAFRA.
- Concerned In-charge of LEC/RNR-EC of the affected Gewog.
- Mangmi/Tshogpa of the affected Gewog/Chiwog.
- Epidemiologist/Expert from NCAH, Serbithang – if required
- Laboratory Officer/Sr. Lab. Technician, NCAH, Serbithang – if required

Note: the team composition shall vary depending on the magnitude of the outbreak which will be decided by the concerned RLDC.

B. Roles of RRT

- As soon as suspected cases are reported, conduct a thorough investigation.
- Recommend Dzongkhag Administration to issue an outbreak declaration order.
- Declare infected and protection zones.
- Implement a ban on the movement of livestock and their products.
- Isolation and treatment of affected animals.
- Disinfection of infected premises using appropriate disinfectants.
- Collect samples from sick animals, conduct rapid field tests if available, and refer samples to the NCAH for further confirmation.
- Conduct surveillance in the infected and protection zones.
- Constantly monitor the outbreak situation in the affected area.
- Mobilize different teams for the response as per their roles.

- Submit the weekly follow-up report on the disease status to Dzongkhag, RLDC, NCAH, DoL, and BAFRA.
- Create awareness for different stakeholders.
- Mobilization of manpower and resources where required.
- Seek the support of the Department/NCAH on any additional fund/logistics.

C. Roles of different teams under RRT

The RRT is divided into different teams as per the mandate of the respective technical sectors involved in disease control measures. The livestock sector will be mainly responsible for surveillance, treatment, outbreak investigation, and logistic support, while BAFRA will be responsible for the quarantine and movement control of susceptible livestock and products. In case there is the requirement of other stakeholders, their support will be sought by the team leader of RRT as and when required. The outbreak of the disease in Tshethar animals shall be dealt with as per the Tshethar guidelines, and through the involvement of the concerned authority.

i. Disease Outbreak Investigation Team (DOIT)

The DOIT shall be responsible for conducting disease outbreak investigation which includes confirming the outbreak, identifying the source of infection and risk assessment. They should be responsible for the identification and establishment of infected premises and declaration of Infected and Protection zones and make key recommendations to improve the control activities based on the disease situation in the area (SOP 1). The team is also responsible for the weekly reporting of the disease status to the NCAH and the Department of Livestock.

The DOIT will be constituted of the following members:

- Veterinary Officers
- Laboratory Technicians
- BAFRA officials

By involving some hired labourers, disposal of dead carcasses and disinfection of contaminated premises and materials shall be carried out (SOP 3). The carcass of animals that died due to LSD should be properly disposed of to avoid the spread of the virus through contamination (SOP 2).

ii. Surveillance Team

The surveillance team ("clean team") is responsible for conducting surveillance activities in the protection zone. The team must be stationed away from the teams implementing response measures in the infected zone.

The surveillance team shall be composed of the following members:

- Veterinary Officer/ Veterinary paraprofessional – team leader
- Laboratory Technician.
- Mangmi/ Tshogpa.

iii. Quarantine and Movement Control Team

The Quarantine and Movement Control Team shall be responsible for enforcement of quarantine and movement control in and out of the infected zone to control and prevent the spread of disease (SOP 5).

The Quarantine and movement control team will be composed of the following members:

- Livestock Regulatory and Quarantine Officer, BAFRA
- Police personnel (optional)

iv. Logistics Team

The main role and responsibility of the logistics team are to ensure that all necessary logistic facilities like PPE, materials and equipment, food/refreshment, and transportation are made available to RRT and to reinforce all essential supplies.

The logistics team shall be composed of the following members:

- RLDC – team leader
- Dzongkhag Livestock Officer.
- Livestock Extension Supervisor.
- Mangmi/ Tshogpa.

D. Operation modality

- Following the report of the disease outbreak in the field, RLDC should decide on the activation of the RRT based on the disease situation. If RRTs are to be activated, it should be done within 24 hours of the confirmation of LSD outbreak.
- RRT should seek the support of the NCAH and the Department of Livestock, as and when required.
- Once the outbreak is under control, the RRT Team Leader should decrease the number of members involved in the LSD outbreak control activities as the main activities of the team will be on the surveillance in the protection zone and other nearby areas. The final deactivation of RRT will be after four weeks from the last case of LSD, however, the duration is subject to change based on risk assessments.

E. Logistics required

Manpower:

- Mobilize additional staffs from nearby centres, RLDC, NCAH, Department and other Dzongkhags and relevant stakeholders.

Vehicle

- Mobilize from Dzongkhags, RLDC, NCAH, projects and other central programmes if required.

Diagnostics

- RLDC/NCAH should facilitate rapid diagnostic kits and other sampling equipment.

Fund

- The fund required for the purchase of diagnostics should be made available by the NCAH and the Department.
- Expenses for the working lunch/refreshment and DSA during the disease containment programme should be arranged by the respective RLDCs and Dzongkhags. If there are no fund provision or insufficient funds, the NCAH and Department should arrange to provide the required funds to the RRT.

Annexure 2: Standard Operating Procedures (SOPs)

SOP 1: Disease outbreak investigation

An outbreak investigation is a systematic procedure to help identify the causes and sources of the epidemic to control the existing outbreak(s) and its prevention in the future.

A. Purpose

- To identify the causes and sources of disease outbreak.
- To identify measures to prevent the further spread of disease.
- To control and contain the existing disease outbreak.

B. Scope

- This SOP outlines the general principles and steps for the investigation of the LSD outbreak in the field.

C. Users/targets

- Veterinary Officers and Veterinary paraprofessionals
- Rapid Response Teams

D. Team composition

- Epidemiologist/ Veterinary Officer
- Laboratory Technician
- BAFRA official

E. Materials and equipment - quantities required should be decided by the team.

- Disposable gloves
- Gumboot
- Apron
- Shoe covers
- Scissors
- Forceps
- VTM
- Cotton
- Vacutainer with anticoagulant
- Vacutainer without anticoagulant
- Adapter for blood collection
- 16G needles
- Marker pen
- Cool box
- Bio-hazard bags

- Disinfectants – Virkon-S/ Bleaching powder/ Sodium hydroxide
- Antiseptic hand wash
- Ice pack
- GPS
- Laboratory sample submission form
- Written Instructions/Printed SOP
- Notebook and pens

F. Steps for Investigation

- *Pre-investigation preparation*
  - Formation of an investigation team and planning the response activities among team members.
  - Discuss each person's roles and responsibilities.
  - Arrangement of logistics – food, vehicle, etc.
  - Preparation of checklist and packaging of materials and equipment enlisted above.

- *Gather preliminary information*

The following information needs to be collected/shared by the team before their departure:

- The number of households with the cases.
- Farmers' name and phone number (if available).
- Name of village(s), Gewog, Dzongkhag.
- Type of rearing and number of animals (stall-fed, open grazing, tethering, etc.).
- Date and time of the report of an outbreak from farmer to LEC/ DVH.
- Date and time of report from LEC/ DVH to RLDC/NCAH.
- Date and time of the visit by veterinarian or field staff.
- Contact details of the field staff.
- Provide information about the team's visit to the outbreak area: date and time of visit.

- *Field investigation*

Background information to collect:

- Farm and village background information.
- Different animal categories and numbers (herd size).
- Farm type and husbandry practices.
- Information regarding inter-mixing of susceptible animals.
- General information regarding the introduction of any new animals.
- General information regarding buying and selling of any livestock and livestock products.
- General information about the affected village/farm.
- Information about the prevalence of vectors.

- Collect other information such as geo-coordinates, altitude, road network, communal pasture, etc.

Baseline morbidity, mortality, and clinical signs:

- General information of the present disease outbreak such as the number of households affected, the population at risk, livestock population in the surrounding villages, etc.
- Determine baseline mortality for a period (week or month) before the outbreak.
- Record of the daily morbidity and mortality figures in the herd/village.
- Record of the detailed clinical signs observed.

Bio-security arrangements:

- Describe the bio-security arrangement of the farm or herd.
- Mixing of different groups including possible contact with wild animals.

Feeding and management:

- Describe the grazing system followed including whether the animals are grazed in their private land or communal pastures or tsamdros.
- Describe feed sources.
- Describe the housing type and the bedding materials used in the shed.
- Describe water source.

Wild animals:

- Determine the presence of any wild animals in the area.
- Determine whether there are any suspected LSD cases in the wild animals around the locality.

Vaccination history

- Verify whether the animals in the affected herd/villages are vaccinated against LSD when imported against LSD and other diseases. Also record details about vaccination against other diseases.

- *Laboratory investigation*
  - Put on proper PPE (apron, gloves, gumboots, and shoe cover).
  - Carry out a physical examination of the sick animals and check for lesions.
  - Collect swabs and blood samples and transport them to the laboratory (SOP 4).
  - LSD specific laboratory tests will be performed at designated laboratories for confirmation of the infection (SOP 6).

- *Characterize the outbreak.*
  - Establish or verify the outbreak.
  - Provisional diagnosis made on clinical signs and epidemiological patterns.
  - Provisional disease control measures should be in place before the confirmatory diagnosis is made.

- *Establish the case definition for LSD.*
- *Describe the outbreak in terms of cases, time, and place.*
  - When was the index case?
  - What is the exact period of the outbreak?
  - Given the diagnosis, what is the probable period of exposure?
  - Is the outbreak most likely to be point source or propagated or both?
  - Time: draw the epidemic curve by plotting cases against the timeline.
  - Animal (attack rates, risks, etc.)
    - Any differences in the attack rates among different herds, species, etc.
    - Which groups (cattle or buffaloes) have the highest and which have the lowest attack rate?
    - Any difference in the attack rate among the different age groups of susceptible animals?
  - Place (plot the location of the outbreak on a map with physical characteristics such as road, water bodies, mountains, infrastructures, etc.)
    - What are the geographical distributions of the cases?
    - What is the pattern of the cases among different species in different management systems?
    - Whether the affected farm is close to the international borders, national highways, migratory routes, or other spatial risk factors?

- *Develop a hypothesis based on the pattern of disease (animal, time, and place)*
  - Source of disease outbreak – forward and backward contact tracing.
  - Mode of transmission.
  - Whether the outbreak is a common source or propagating.
  - If a common source, whether it is a point or multiple exposures.
  - What are the risk factors associated with the problem?

SOP 2: Disposal of carcasses

A. Purpose

To have standard procedures for the safe disposal of LSD infected carcasses and infected materials.

B. Scope

This SOP describes procedures for site selection and burial of carcasses safely to avoid the spread of the virus through contamination.

C. Users

- Veterinary Officer/Veterinary paraprofessionals

D. Manpower

- Livestock Regulatory and Quarantine Officials, BAFRA.
- Hired labourers.
- Animal Owner.
- Concerned Gewog livestock in-charge.

E. Materials/Equipment required

- Hand gloves.
- Face masks.
- Apron (disposable).
- Gumboot.
- Disinfectants – Calcium carbonate (lime)/sodium hypochlorite (2-3%).
- Digging tools: spades, crowbars, pickaxe, shovel, etc.

F. Procedures

- Select an appropriate site for carcass burial. The site should be away from the water sources, residential areas, livestock facilities, pastures and other establishments in the vicinity. Preferably it should be away from any footpaths or roads leading to the site.
- Prepare a pit with sufficient width to accommodate the carcass with a minimum depth of 2 meters considering the size of the carcass.
- Wear an apron, face masks, gumboot, and hand gloves before handling the carcasses.
- Drop the carcasses into the pit and dispose of the hand gloves, face mask, and apron into the pit.
- Cover the carcasses with soil, 400 mm is suggested, and add an unbroken layer of lime (calcium carbonate). Do not spray lime directly onto the carcasses as it will slow the decomposition process.
- Close the pit with sufficient soil and make a heap over the site.
- Put a layer of lime over the soil.

- The disposal site should be secured by putting stones, thorns, logs, etc.
- Disinfect vector breeding sites such as standing water sources, slurry, manure and vector holding areas.
- All tools, utensils and equipment used for burial should be thoroughly cleaned and disinfected with disinfectant solutions (2-3%sodium hypochlorite).
- The animal handlers should thoroughly clean and disinfect themselves before leaving the burial site.

SOP 3: Disinfection of contaminated premises, materials, and personnel

A. Purpose

To have a standard procedure for effective disinfection of contaminated premises and materials.

B. Scope

The document describes procedures for the disinfection of contaminated materials and premises.

C. Users

- Veterinarians/ Veterinary paraprofessionals.

D. Manpower

- Livestock Regulatory and Quarantine Officials, BAFRA.
- Hired labourers.
- Animal Owner.
- Concerned Gewog livestock in-charge.

E. Materials/ Equipment required

- Hand gloves.
- Apron (disposable).
- Gumboots.
- Buckets.
- Mugs/jugs.
- Water.
- 2% Phenol, 2% Virkon-S, 2-3%Sodium hypochlorite, 1:33 dilution Iodine compounds, etc.
- Sprayer (if available).

F. Procedures

- Prepare 2% Virkon, phenol (2%), sodium hypochlorite (2–3%), iodine compounds (1:33 dilution), or quarternary ammonium compounds (0.5%).
- Collect the bedding materials and bury them with carcasses if it is in small quantities, or collect and burn it in a pit if in larger quantities.
- Contaminated premises should be mechanically cleaned and disinfected thoroughly with the disinfectant; allow a contact time of 2 – 3 hrs.
- Disposable items including used PPEs (gloves, apron, mask, shoe cover) must be buried in a pit or incinerated.
- While leaving the infected premises, the personnel and vehicles should be thoroughly disinfected.

- All tools, utensils, equipment used for burial should be thoroughly cleaned and disinfected with a disinfectant solution.

SOP 4: Sample collection for LSD diagnosis

A. Purpose

This document outlines the methods for sample collection, storage and transportation from the LSD affected animals to the laboratory for detection of LSDV.

B. Scope

This procedure can be applied in any kind of sample collection from LSD affected animals for detection of LSDV PCR technology.

C. Equipment/materials

- General materials
  - Labels and permanent markers.
  - Data collection forms, pens, clipboards.
  - Sharps bin for needle and scalpel disposal.
  - Autoclavable disposal bags.
  - Forceps.
  - Swabs.
  - Sterile container with PBS/VTM.
  - Disinfectant (2% virkon/bleach).

- Personal Protective Equipment
  - Dedicated clothing (coveralls).
  - Rubber boots.
  - Boot covers.
  - Gloves.
  - Facemasks.
  - Safety glasses for eye protection.
  - Hand disinfectants.
  - Boot disinfectant.

- Materials for sample transport
  - Primary containers/sterile tubes/vials (leakproof and clearly labelled)
  - Absorbents
  - Coolbox /Styrofoam box filled with cooling materials (ice, frozen water bottles, or cold packs)

- Sampling materials for live animals
  - Materials for restraining animals.
  - Cotton wool and disinfectant to clean sampling site.

- Sterile vacutainers (10 ml) without anticoagulant (red stoppers) for serum collection.
- Sterile vacutainers (10 ml) with EDTA (purple stoppers) for whole-blood collection.
- Vacutainer holders and vacutainer needles or 10-20 ml syringes.
- Swabs.
- Injectable local anaesthetics, disposable biopsy punches or scalpels and suture. material if full-thickness skin samples are to be collected from live animals.

- Materials for post-mortem sampling
  - Sample racks or cryo-boxes for cryo-vials.
  - Sterile cryovials of appropriate size for organ collection (can be prefilled with medium for sample preservation if the cold chain is not optimal).
  - Knives, knife sharpeners, shears, scalpels and blades, forceps and scissors.
  - Containers with disinfectant for disinfecting knives, scissors, etc. between organs and between animals, to avoid cross-contamination.
  - Securely sealable plastic pots filled with 10% neutral buffered formalin (1:10 organ volume: formalin volume ratio).
  - Appropriate materials for carcass disposal.

D. Procedures

- Sampling from Live animals
  - Swabs from nodular fluids/discharges from the nasal, mouth and ocular sites and preserve in VTM or PBS.
  - Skin Nodular Lesions – Skin scrapings/ Scabs.
    Collect skin biopsy from skin nodules or scabs (2-4 numbers) preferably from upper body surface using sterile forceps or swabs. Place it in a sterile container with viral transport medium (VTM) or sterile phosphate buffer saline (PBS) and store it at refrigerated temperature (4°C) and ship immediately in a cool box with ice. If the shipping period is >48 hrs, store in -80°C.
    Use local ring block-anaesthesia if you surgically collect full-thickness samples from skin lesions – disposable biopsy punches 16 to 17 mm in diameter can be used.
  - Whole Blood/Serum
    Collect a minimum of 5 ml of blood from the jugular or tail vein (coccygeal vein) in sterile vacutainers (10 ml) with EDTA (purple stoppers) and store it at refrigerated temperature (4°C) until shipping in ice. For serum, collect blood in vacutainer tubes without anticoagulant, stand to separate serum and store at 4°C.

- Sampling from Dead animals
  In dead animals, samples should include skin lesion nodules, lung lesions (including normal tissue), lymph nodes - mediastinal lymph nodes and other organs with nodular lesions.

- Histopathology
  For histopathology, preserve the tissues in 10% formalin.

E. Shipment of samples

- Sample Information
  Information and case history should always accompany the samples to the laboratory and should be placed in a plastic envelope on the outside of the shipping container. The sample submission form (See Annexure 3, Form 2) should be filled and submitted to the receiving laboratory along with the samples.

- Sample packaging
  The recommended procedure for packing samples are as follows:
  - Put the samples in a primary container with screw caps and wrap them with paraffin film or adhesive tape individually to prevent leakage of fluid. The wrapping of primary containers should be carried out in clean surroundings. Put the primary container into a watertight, spill-proof secondary container with absorbent cotton wool sufficient to absorb the entire contents of the primary container (in cases of leakage).
  - Place the secondary container in an outer container. This should be a polystyrene foam box covered with a hard box or other appropriate containers (E.g., coolbox).
  - It is recommended that a freezer box/ice packs are put outside the secondary packaging to ensure that all materials are kept cool and not frozen during shipment. These packs should be pre-frozen at – 20 degrees centigrade before packaging.

- Transportation of specimens
  The specimens should be forwarded to the laboratory by the fastest method available. If they can reach the laboratory within 48 hours, samples should be sent refrigerated.

F. Safety

- When samples are taken from live animals, care should be taken to minimize distress to the animal and avoid injury to the animal handlers and sample collector.
- All the materials used for sampling skin tissue should either be autoclaved or safely disposed of.
- Disinfect the sample collection site and change needles, scalpels and gloves.

SOP 5: Quarantine and movement control

A. Purpose

To have a standard procedure for effective quarantine and movement control during LSD outbreak(s).

B. Scope

The document describes procedures for quarantine and movement control to contain the disease.

C. Users

- Regulatory Officers (BAFRA officials).
- Police personnel.

D. Materials/Equipment required

- Barrier tape.
- Signboard.
- Basic PPEs (Masks, Gloves).
- Disinfectants.
- Sprayer.
- Foot dip.
- Register.

E. Procedures

- Restrict the movement of all the susceptible animals (cattle and water buffaloes) and their products from the LSD outbreak areas and/or routing through an affected area.
- Ban on importation of live susceptible animals and their products into/routing through an area affected with LSD.
- The slaughter and sale of meat from susceptible animals should be banned in the outbreak area(s).
- The animals in the affected herd should be confined within their shed and avoid mixing with other healthy animals. These animals should be provided proper treatment.
- The team shall seek local government support for the enforcement of bans and restrictions.

SOP 6: Detection of Capripox Virus (including LSDV) by Real-time PCR

A. Purpose

The purpose of this procedure is rapid detection of the capripoxvirus (CaPV) genome from clinical samples by the real-time polymerase chain reaction (PCR) technique.

B. Scope

This procedure can be applied in any kind of ruminant clinical samples such as EDTA-blood, serum and tissue homogenates and cell culture supernatants. It is to support the diagnosis of CaPV in Bovine, Caprine and Ovine species using real-time PCR test.

C. Test principles

In RT-PCR, the DNA is used as a template for exponential amplification using PCR. RT-PCR is currently the most sensitive method of RNA/DNA detection available. It can be used for both qualitative analysis and quantitative analysis. In Fluorescent End-Point PCR, the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect the fluorescence emission from the fluorophores in a reaction mixture after PCR. It allows detection of the accumulating product without re-opening the reaction tubes after the PCR run. The method was adapted from Bowden et al., 2008 for the detection of all CaPV including the Lumpy skin disease virus. The assay uses a dual labelled fluorogenic (TaqMan®) probe and primers specific to *Capripoxvirus*.

D. Equipment and Materials

- Equipment
  - QuantStudio-5/real time PCR machine.
  - MINI spin / micro-centrifuge for Eppendorf tubes.
  - Refrigerated centrifuge, PK-121R, Thermo Electron Corporation.
  - Heating block/water bath.
  - Freezers (- 20^{o}C).
  - Freezer (- 80^{o}C).
  - Fridge (2 – 8^{o}C).
  - Vortex.
  - Bio-Safety Cabinet, Class –II.

- Materials
  - Single-channel pipette 1-10µl.
  - Single-channel pipette 2-20µl.
  - Single-channel pipette 20-200µl.
  - Single-channel pipette 100-1000µl.
  - Micropipette tips of 1-200 and 200-1000 µl, sterile.

- Micropipette tips with the aerosol resistant filter of 1-10, 2-20, 20-200 and 100- 1000 µl, sterile.
- Microcentrifuge tubes of volumes 0.2, 0.5, 1.5, and 2 ml, sterile.
- DNA extraction kit, Qiagen
- Ethanol 100%, Merck
- AgPath-ID, One-Step RT-PCR Reagents, Catalogue number: 4387391
- Distilled H2O, sterile, PCR grade.
- Positive control; Known diluted ASF sample
- Negative controls: Nuclease free water
- Latex or nitrile gloves
- Biohazard bag

- Reagents
  - Forward primer CaPV074F1 5'-AAA ACG GTA TAT GGA ATA GAG TTG GAA-3'
  - Reverse primer CaPV074R1 5'-AAA TGA AAC CAA TGG ATG GGA TA-3
  - Probe: CaPV074P1 5'-FAM-TGG CTC ATA GAT TTC CT-MGB/NFQ-3'
  - Positive Control: reference LSDV strain and reference CaPV strain
  - Negative control: Nuclease free water

E. Sample

- Scab, skin nodules, nasal swab, mouth swab, ocular swab, blood in EDTA used for extraction of DNA.

F. Procedures

- Extraction of DNA (Template DNA)
  - Isolate a suitable piece of tissue and place it in a UV-crosslinked 1.5mL tube.
  - Add 180 ml Buffer ATL and 20ml Proteinase K and vortex.
  - Place in the 55 oC incubator for 3 hours or overnight.
  - Remove from incubator, vortex, add 200ml Buffer AL and vortex.
  - Place in a heat block at 70 oC for 10 minutes.
  - Add 200ml 100% Ethanol and transfer the entire volume onto the spin column.
  - Centrifuge at 8000 rpm for 1 minute; discard flow-through.
  - Add 500ml Buffer AW1 and centrifuge at 8000 rpm for 1 minute, discard flowthrough.
  - Add 500ml Buffer AW2 and centrifuge at 13000 rpm for 3 minutes, discard flowthrough.
  - Place spin column on UV-crosslinked 1.5mL tube, add 200ml buffer AE. Let sit for 1 minute, then centrifuge at 8000 rpm for 1 minute. Repeat and then combine flowthroughs for a total volume of 400ml.
  - Store the extracted DNA at 4^{o}C for immediate use, otherwise at -80^{o}C for long term

The RNA extraction kit from Qiagen can be used for DNA extraction in cases of non-availability of the DNA extraction kit.

G. DNA amplification

- Master mix preparation

In a sterile 1.5 ml Microcentrifuge tube, prepare the PCR reaction mixtures described below for the number of samples to be assayed. The tables below show master mix preparation:

- Using qPCR Master Mix-Path ID.

Reagent	**Final Conc**	**µL per reaction**
DNAse/RNAse free water		6.7
Forward primer- 20 µM	400 nM	0.4
Reverse primer-20 µM	400 nM	0.4
Probe- 10 µM	250 nM	0.5
2X qPCR Master Mix-Path ID	1X	10
Total volume		18 µL

(Or)

- Using AmpliTaq Gold DNA Polymerase.

Reagent.	**Final Conc**	**µL per reaction**
DNAse/RNAse free water		2.7
Forward primer- 20 µM	400 nM	0.4
Reverse primer-20 µM	400 nM	0.4
Probe- 10 µM	250 nM	0.5
2X RT-PCR buffer mix	2X	12.5
AmpliTaq Gold DNA Polymerase	1X	1
ROX reference dye		0.5
Total volume		18 µL

Add 18 µl of the PCR reaction mix to the required number of 0.2 ml optical PCR tubes including the positives controls and the negative controls, adding at least one additional sample to minimize pipetting mistakes.

- Sample addition
  - Add 2µl of DNA template to each PCR tube. Include positive control (2 µl of LSDV/CaPV DNA) and negative control (2 µl of nuclease-free water)
  - After the addition of the template, close the reaction tube and spin down the PCR mix.
  - Place all tubes in an automated real-time thermocycler.
  - Run the incubation program detailed below.

- PCR cycle condition
  - 1X 95°C 10 min,
  - 45X 95°C 15 sec, 60°C 45 sec (Read)
  - Program the fluorescence collection in FAM channel and quencher as MGB/NFQ

H. Analysis and interpretation of results

In a positive sample, a sigmoid-shaped amplification curve will be obtained, indicating the cycles number versus reading fluorescence level, where the Ct value will be under 40.
A negative sample will maintain the fluorescence profile under the background fluorescence level and the equipment will not report any Ct value. Therefore, a negative sample will show a Ct value ≥40.

I. Critical points

Because PCR is a highly sensitive technique, the most critical point along all the analysis procedure is the considerable risk of carry-over contamination, and the false-positive results that could be obtained in this situation. The contamination could be due to the ASFV itself present in the positive analysed samples or the positive controls included in the DNA extraction procedure; also, it could be due to ASFV DNA obtained after amplification of a previous PCR. Personnel working on PCR must follow and carry out some strict work rules to minimize the contamination risk associated with the PCR technique:

- All steps of sample analysis by PCR should be performed in separate locations, using equipment and material-specific for each one: sample preparation, DNA extraction, PCR mix preparation, and removal of PCR products.
- Personnel must work always with clean nitrile or latex gloves in the PCR laboratory.
- Change of gloves whenever personnel go into a different PCR area,
- Tubes containing amplified product should never be opened and manipulated in another laboratory distinct to that exclusively assigned to their analysis by electrophoresis, where they will be discarded.

J. Waste disposal

All the wastes should be discarded after being autoclaved.

Annexure 3: Standard Forms

Form 1: Flash report form.

ད་དམག་ལན་འབག་གཞུང་།
གཙོ་མ་དང་ནགས་ཚལ་ལན་ཁག།ཁོར་རོལ་ལས་ཁང་།
ROYAL GOVERNMENT OF BHUTAN
Ministry of Agriculture and Forests
Department of Livestock
Thimphu

Flash Report Form for Animal Disease Outbreak Reporting

Sl. No.	Parameters	Information					
1	Name of owner (if applicable)/ Community						
2	Location (geo-coordinates)	Latitude:		Longitude:			
3	Village						
4	Gewog						
5	Dzongkhag						
6	Date of outbreak						
7	Date of report to LEC/RNR-EC/DVH (by the owner or community)						
8	Date of report by DVH to RLDC/NCAH						
9	Disease suspected						
10	Ear notch status (for canine rabies)	Yes		No			
11	No. of case(s), excluding death	Species	Breed	Young		Adult	
				Male	Female	Male	Female
12	No. of death(s)	Species	Breed	Young		Adult	
				Male	Female	Male	Female
13	Vaccination status (last date/month)						
14	No. of household(s) affected						
15	No. of susceptible animals in the locality (species-wise)						
16	Probable source of outbreak						
17	Contact person in the village (name and phone no.)						
18	Control measures implemented						
Submitted by:							
Name		Designation		Centre			

Form 2: Sample recording and the submission form

Dzongkhag: …….. Gewog: …….. Date: ……..

Sl. No.	Sample collector Details		Farm Details									Animal Details							Sample Details					
	Agency	Designation (Ph. No)	Owner name	Farm type	Farm location (GPS)	Farm size	Domesticated/wild animal	Village	Gewog	District	Contact details	Species	Age	Sex	Breed	Health status (Sick/ dead/ Normal)	Clinical History	Treatment details	Sample ID	Collection date	Type of sample	Pooled sample	Transport media/ preservatives	Test requested

Annexure 4: LSD risk zones, gewog-wise

Risk categories:

High – Red
Medium – Orange
Low – Green

Dzongkhag	Gewog	Risk	Dzongkhag	Gewog	Risk
Bumthang	Chhoekhor	Low	Samdrup Jongkhar	Phuentshogthang	High
Bumthang	Chhumig	Low	Samdrup Jongkhar	Samrang	High
Bumthang	Tang	Low	Samdrup Jongkhar	Serthig	High
Bumthang	Ura	Low	Samdrup Jongkhar	Wangphu	Medium
Chhukha	Bjagchho	Medium	Samtse	Doomtoed	Medium
Chhukha	Bongo	Medium	Samtse	Dophuchen	Medium
Chhukha	Chapchha	Low	Samtse	Duenchhukha	Medium
Chhukha	Darla	High	Samtse	Namgyalchhoeling	High
Chhukha	Doongna	Medium	Samtse	Norboogang	High
Chhukha	Geling	Medium	Samtse	Norgaygang	High
Chhukha	Getana	Medium	Samtse	Pemaling	High
Chhukha	Loggchina	Medium	Samtse	Phuentshogpelri	High
Chhukha	Maedtabkha	Medium	Samtse	Samtse	High
Chhukha	Phuentshogling	High	Samtse	Sang-Ngag-Chhoeling	High
Chhukha	Samphelling	High	Samtse	Tading	High
Dagana	Dorona	Medium	Samtse	Tashichhoeling	High
Dagana	Drukjeygang	Medium	Samtse	Tendruk	High
Dagana	Gesarling	Medium	Samtse	Ugyentse	High
Dagana	Gozhi	Medium	Samtse	Yoseltse	High
Dagana	Karmaling	High	Sarpang	Chhudzom	Medium
Dagana	Karna	Medium	Sarpang	Chhuzanggang	High
Dagana	Khebisa	Medium	Sarpang	Dekiling	High
Dagana	Largyab	Low	Sarpang	Gakiling	High
Dagana	LhamoiDzingkha	High	Sarpang	Gelegphu	High
Dagana	Nichula	High	Sarpang	Jigme Chhoeling	Medium
Dagana	Tashiding	Medium	Sarpang	Samtenling	High
Dagana	Tsenda-Gang	Medium	Sarpang	Senggey	High
Dagana	Tseza	Low	Sarpang	Serzhong	Medium
Dagana	Tshangkha	Medium	Sarpang	Shompangkha	High
Gasa	Khamaed	Low	Sarpang	Tareythang	High
Gasa	Khatoed	Low	Sarpang	Umling	High
Gasa	Laya	Low	Thimphu	Chang	Low

Gasa	Lunana	Low	Thimphu	Darkarla	Low
Haa	Bji	Low	Thimphu	Ge-nyen	Low
Haa	Gaki-ling	Medium	Thimphu	Kawang	Low
Haa	Kar-tshog	Low	Thimphu	Lingzhi	Low
Haa	Samar	Low	Thimphu	Maedwang	Low
Haa	Sangbay	Medium	Thimphu	Naro	Low
Haa	Uesu	Low	Thimphu	Soe	Low
Lhuentse	Gangzur	Low	Trashi Yangtse	Boomdeling	Low
Lhuentse	Jarey	Low	Trashi Yangtse	Jamkhar	Low
Lhuentse	Khoma	Low	Trashi Yangtse	Khamdang	Medium
Lhuentse	Kurtoed	Low	Trashi Yangtse	Ramjar	Medium
Lhuentse	Maedtsho	Low	Trashi Yangtse	Toetsho	Medium
Lhuentse	Maenbi	Low	Trashi Yangtse	Tongmajangsa	Medium
Lhuentse	Minjey	Low	Trashi Yangtse	Yalang	Medium
Lhuentse	Tsaenkhar	Low	Trashi Yangtse	Yangtse	Medium
Monggar	Balam	Low	Trashigang	Bartsham	Medium
Monggar	Chagsakhar	Low	Trashigang	Bidoong	Medium
Monggar	Chhaling	Low	Trashigang	Kanglung	Low
Monggar	Dramedtse	Low	Trashigang	Kangpar	Medium
Monggar	Drepoong	Low	Trashigang	Khaling	Medium
Monggar	Gongdue	Medium	Trashigang	Lumang	Medium
Monggar	Jurmed	Medium	Trashigang	Merag	Medium
Monggar	Kengkhar	Low	Trashigang	Phongmed	Medium
Monggar	Monggar	Low	Trashigang	Radi	Medium
Monggar	Na-Rang	Low	Trashigang	Sagteng	Medium
Monggar	Ngatshang	Low	Trashigang	Samkhar	Low
Monggar	Saling	Low	Trashigang	Shongphu	Medium
Monggar	Shermuhoong	Low	Trashigang	Thrimshing	Medium
Monggar	Silambi	Medium	Trashigang	Udzorong	Low
Monggar	Thang-Rong	Low	Trashigang	Yangnyer	Low
Monggar	Tsakaling	Low	Trongsa	Draagteng	Low
Monggar	Tsamang	Low	Trongsa	Korphu	Low
Paro	Dokar	Low	Trongsa	Langthil	Low
Paro	Dopshar-ri	Low	Trongsa	Nubi	Low
Paro	Doteng	Low	Trongsa	Tangsibji	Low
Paro	Hoongrel	Low	Tsirang	Barshong	Medium
Paro	Lamgong	Low	Tsirang	Doonglagang	Medium
Paro	Loong-nyi	Low	Tsirang	Gosarling	Medium
Paro	Nagya	Low	Tsirang	Kilkhorthang	Medium

Paro	Sharpa	Low	Tsirang	Mendrelgang	Medium
Paro	Tsento	Low	Tsirang	Patshaling	Medium
Paro	Wangchang	Low	Tsirang	Phungtenchhu	Medium
Pema Gatshel	Chhimoong	Medium	Tsirang	Rangthangling	Medium
Pema Gatshel	Chhoekhorling	High	Tsirang	Semjong	Medium
Pema Gatshel	Chongshing	Medium	Tsirang	Sergithang	Low
Pema Gatshel	Dechhenling	Medium	Tsirang	Tsholingkhar	Medium
Pema Gatshel	Dungmaed	Medium	Tsirang	Tsirang Toed	Low
Pema Gatshel	Khar	Medium	Wangdue Phodrang	Athang	Low
Pema Gatshel	Nanong	Medium	Wangdue Phodrang	Bjenag	Low
Pema Gatshel	Norboogang	High	Wangdue Phodrang	Dangchhu	Low
Pema Gatshel	Shumar	Medium	Wangdue Phodrang	Darkar	Low
Pema Gatshel	Yurung	Medium	Wangdue Phodrang	Gangteng	Low
Pema Gatshel	Zobel	Medium	Wangdue Phodrang	GaseTshogongm	Low
Punakha	Barp	Low	Wangdue Phodrang	GaseTshowongm	Low
Punakha	Chhubu	Low	Wangdue Phodrang	Kazhi	Low
Punakha	Dzomi	Low	Wangdue Phodrang	Nahi	Low
Punakha	Goenshari	Low	Wangdue Phodrang	Nyishog	Low
Punakha	Guma	Low	Wangdue Phodrang	Phangyuel	Low
Punakha	Kabisa	Low	Wangdue Phodrang	Phobji	Low
Punakha	Lingmukha	Low	Wangdue Phodrang	Rubesa	Low
Punakha	Shelnga-Bjemi	Low	Wangdue Phodrang	Saephu	Low
Punakha	Talog	Low	Wangdue Phodrang	Thedtsho	Low
Punakha	Toedpaisa	Low	Zhemgang	Bardo	Medium
Punakha	Toedwang	Low	Zhemgang	Bjoka	Medium
Samdrup Jongkhar	Dewathang	High	Zhemgang	Goshing	Medium
Samdrup Jongkhar	Gomdar	Medium	Zhemgang	Nangkor	Medium
Samdrup Jongkhar	Langchenphu	High	Zhemgang	Ngangla	High
Samdrup Jongkhar	Lauri	High	Zhemgang	Pangkhar	High
Samdrup Jongkhar	Martshala	Medium	Zhemgang	Shingkhar	Medium
Samdrup Jongkhar	Orong	High	Zhemgang	Trong	Medium
Samdrup Jongkhar	Pemathang	High			

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