



manual

LUMPY SKIN DISEASE

A field manual for veterinarians

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Foreword

Lumpy skin disease (LSD) was for long restricted to sub-Saharan Africa. However, over the past decades it has slowly invaded new territories, sweeping first into the Middle East and Turkey, and, since 2015, into most of the Balkan countries, the Caucasus, and the Russian Federation, where the disease continues to spread despite implemented prevention and control efforts. The disease is having dramatic effects on rural livelihoods, which are strongly dependent on cattle, with significant income losses for affected farmers. Consequences are also devastating at national level since the presence of the disease has triggered strict trade restrictions. The risk of imminent contagion of neighbouring countries is very high.

In the current situation, veterinary services from affected and at-risk countries in the Middle East and Europe are confronting the disease for first time. Official veterinarians, cattle farmers and others along the value chain are therefore unfamiliar with the disease's clinical presentation, its transmission routes, and available prevention and control options. This manual is aimed at filling these gaps, principally with regard to the first line of defence, i.e. those working in the field, who are most likely to encounter the disease.

The authors would like to express their sincere thanks to the global scientific community contributing to LSD research, as well as to international organizations working in this field, such as the World Organisation for Animal Health (OIE), the European Commission and the Directorate-General for Health and Food Safety (DG SANTE), the European Food Safety Authority (EFSA), the European Commission for the Control of Foot-and-Mouth Disease (EuFMD), the International Atomic Energy Agency (IAEA) and national and international reference laboratories. Finally, we would like to express our gratitude to all recently affected countries for sharing their experiences and helping us to describe the best practices available to control and eradicate LSD.

The manual is enriched by pictures kindly provided by a number of excellent international photographers. FAO would like to thank Stephen Ausmus, Tsviatko Alexandrov, Kris de Clercq, Bernard Dupont, Ignacio Ferre Pérez, Douw Grobler, the National Food Agency of Georgia, the Nottingham School of Veterinary Medicine, Alfons Renz, J.C.A. Steyl and Eeva Tuppurainen for offering their photographs for our use. The illustrations were created by Tsviatko Alexandrov (Figure 2) and Mirko Bruni (Figure 1).

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FAO welcomes any feedback and comments.

Andriy Rozstalnyy

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Acronyms

ADR	International Carriage of Dangerous Goods by Road
CaPV	Capripoxvirus
DIVA	Differentiation of Infected from Vaccinated Animals
EFSA	European Food Safety Authority
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EMPRES	Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases
EMPRES-i	EMPRES Global Animal Disease Information System
EuFMD	European Commission for the Control of Foot-and-Mouth Disease
FAO	Food and Agriculture Organization of the United Nations
FMD	Foot-and-mouth disease
GEMP	Good Emergency Management Practice
GPS	Global Positioning System
GTP	Goat pox
GTPV	Goat pox virus
IAEA	International Atomic Energy Agency
IATA	International Air Transport Organization
IFAT	Indirect fluorescent antibody test
IPMA	Immunoperoxidase monolayer assay
LSD	Lumpy skin disease
LSDV	Lumpy skin disease virus
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PPE	Personal protective equipment
SPP	Sheep pox
SPPV	Sheep pox virus
TAD	Transboundary Animal Disease

Introduction

Lumpy skin disease is a vector-borne pox disease of domestic cattle and Asian water buffalo and is characterized by the appearance of skin nodules. Endemic across Africa and the Middle East, the disease has, since 2015, spread into the Balkans, the Caucasus and the southern Russian Federation. Outbreaks of LSD cause substantial economic losses in affected countries, but while all stakeholders in the cattle industry suffer income losses, poor, small-scale, and backyard farmers are hit hardest. The disease impacts heavily on cattle production, milk yields, and animal body condition. It causes damage to hides, abortion, and infertility. Total or partial stamping-out costs add to direct losses. Indirect losses stem from restrictions on cattle movements and trade.

In addition to vectors, transmission may occur through consumption of contaminated feed or water, direct contact, natural mating or artificial insemination. Large-scale vaccination is the most effective way of limiting the spread of the disease. Effective vaccines against LSD exist and the sooner they are used the less severe the economic impact of an outbreak is likely to be.

The purpose of this manual is to enhance awareness of LSD and to provide guidance on early detection and diagnosis for private and official veterinary professionals (in the field and in slaughterhouses), veterinary paraprofessionals and laboratory diagnosticians.

The field manual comprises a general description of LSD, including clinical signs, geographic distribution, epidemiology, host range, and transmission pathways. It then moves chronologically from detection of cattle showing typical clinical signs of LSD – later referred to as “suspected case(s)” – to the consideration of differential diagnoses, postmortem findings, and laboratory confirmation of field diagnosis. The primary diagnostic tools available for the detection of both virus and antibodies are described, as well as recommendations for sample collection and transport from the field to national or international reference laboratories. The immediate control and eradication actions following a suspected/detected LSD case on a farm are described. Additionally, the manual covers various aspects related to awareness-raising and feasible post-outbreak surveillance.

This manual is one of a series prepared by FAO’s Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (EMPRES) as an aid to preparedness for major transboundary animal diseases (TADs) of livestock. Lumpy skin disease is classified as a TAD due to its significant economic impact on production and local livelihoods, and to the international trade restrictions it entails in affected countries. In addition, LSD can rapidly spread across national borders and reach epidemic proportions, thus requiring regional cooperation in prevention, control and eradication (OIE, 2016).

Epidemiology

Typically, LSD outbreaks occur in epidemics several years apart. The existence of a specific reservoir for the virus is not known, nor is how and where the virus survives between epidemics. Outbreaks are usually seasonal but may occur at any time because in many affected regions no season is completely vector-free.

Presence of growing numbers of naïve (i.e. not immune) animals, abundance of active blood-feeding vectors, and uncontrolled animal movements are usually drivers for extensive LSD outbreaks. The primary case is usually associated with the introduction of new animal(s) into, or in close proximity to, a herd.

Morbidity varies between 2 and 45 percent and the mortality rate is usually less than 10 percent. Susceptibility of the host depends on immune status, age, and breed. Generally speaking, high milk-producing European cattle breeds are highly susceptible compared to indigenous African and Asian animals. Cows with high milk production are usually most severely affected.

Asymptomatic, viraemic cattle are commonly detected among infected animals, experimentally and in the field. In order to stop the disease from spreading, it is therefore essential to consider the possible presence in an affected herd of infected animals showing no visible clinical signs, as such animals are capable of transmitting the virus via blood-feeding vectors. The movement of unvaccinated/not immune cattle from infected regions poses a major risk of contagion.

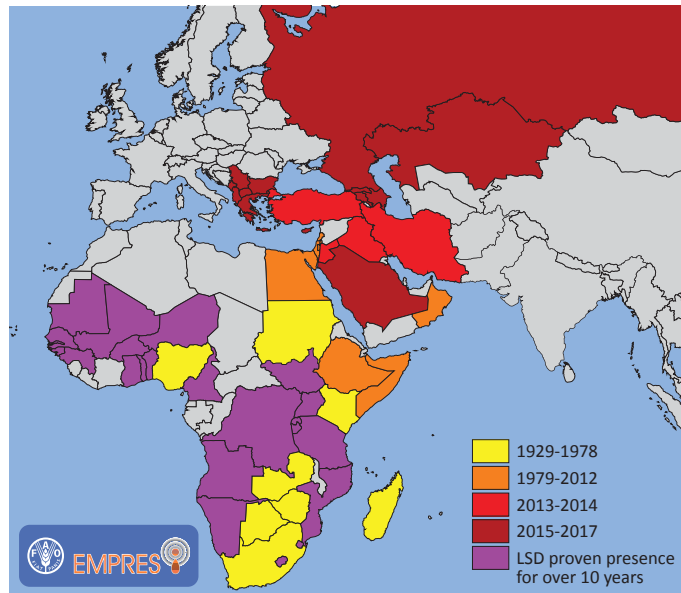
CAUSATIVE AGENT

Lumpy skin disease is caused by the lumpy skin disease virus (LSDV), a member of the genus *Capripoxvirus* (CaPV) within the family *Poxviridae*. Lumpy skin disease virus shares the genus with sheep pox virus (SPPV) and goat pox virus (GTPV), which are closely related, but phylogenetically distinct. There is only one serological type of LSDV, and LSD, SPP and GTP viruses cross-react serologically. The large, double-stranded DNA virus is very stable, and very little genetic variability occurs. Therefore, for LSDV, farm-to-farm spread cannot be followed by sequencing the virus isolates, as is done with other TADs, e.g. foot-and-mouth disease (FMD).

GEOGRAPHIC DISTRIBUTION

Lumpy skin disease is widespread and endemic throughout Africa, excluding Algeria, Morocco, Tunisia and Libya. Since 2013, LSD has swept throughout the Middle East (Israel, the Palestinian Autonomous Territories, Jordan, Lebanon, Kuwait, Saudi Arabia, Iraq, Iran, Oman, Yemen, United Arab Emirates and Bahrain). In 2013, LSD also spread to Turkey, where it is currently endemic. This was followed by outbreaks in Azerbaijan (2014), Armenia (2015) and Kazakhstan (2015), the southern Russian Federation (Dagestan, Chechnya, Krasnodar Kray and Kalmykia) and Georgia (2016). Since 2014, LSD has advanced into the

FIGURE 1
Countries that have reported LSD



The outbreaks in the Russian Federation have been limited to regions within and next to the northern Caucasus.

Source: OIE WAHID and EMPRES-i, 2017

northern part of Cyprus, Greece (2015), Bulgaria, the Former Yugoslav Republic of Macedonia, Serbia, Montenegro, Albania and Kosovo (2016). Currently there is an increased risk of LSD reaching Central Asia, Western Europe and Central-Eastern Europe.

SUSCEPTIBLE HOSTS

Lumpy skin disease is host-specific, causing natural infection in cattle and Asian water buffalo (*Bubalus bubalis*), although the morbidity rate is significantly lower in buffalo (1.6 percent) than in cattle (30.8 percent) (El-Nahas *et al.*, 2011). 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*). The susceptibility of wild ruminants or their possible role in the epidemiology of LSD is not known. Lumpy skin disease does not affect humans.

TRANSMISSION

The first case of LSD can often be traced to the legal or illegal transfer of cattle between farms, regions or even countries. In fact, 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 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 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 postinfection. 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.

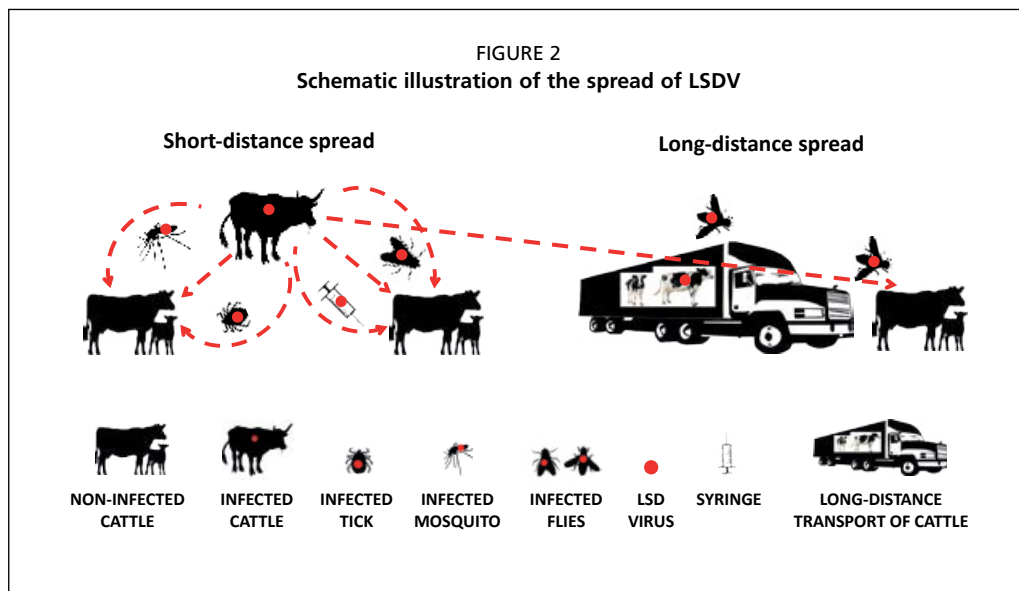


FIGURE 3
Some airborne LSDV vectors



Aedes aegypti mosquito and a common stable fly, *Stomoxys calcitrans*

FIGURE 4
South African Bont Ticks (*Amblyomma hebraeum*) feeding



latrogenic 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.

Clinical signs of lumpy skin disease and postmortem findings

The incubation period in experimentally infected animals varies between four and seven days, but in naturally infected animals it may be up to five weeks. Clinical signs include:

- Lachrymation and nasal discharge – usually observed first.
- Subscapular and prefemoral lymph nodes become enlarged and are easily palpable.
- High fever (>40.5°C) may persist for approximately a week.
- Sharp drop in milk yield.
- Appearance of highly characteristic, nodular skin lesions of 10-50 mm in diameter:
 - The number of lesions varies from a few in mild cases (Figs. 5 and 6), to multiple lesions in severely infected animals (Figs. 7-10).
 - Predilection sites are the skin of the head, neck, perineum, genitalia (Fig. 9), udder (Figs. 14 & 15) and limbs.
 - Deep nodules involve all layers of the skin, subcutaneous tissue and sometimes even the underlying muscles.
 - 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 (Fig. 12).
 - Typically, the centre of the lesion ulcerates and a scab forms on top (Figs. 13, 16 and 17).
 - Skin nodules may persist for several months.
- Sometimes, painful ulcerative lesions develop in the cornea of one or both eyes, leading to blindness in worst cases (Fig. 11).
- 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, subcutaneous lesions are clearly visible after the animal is skinned.

In a postmortem examination, pox lesions can be found throughout the entire digestive and respiratory tracts and on the surface of almost any internal organ (Fig. 18).

FIGURE 5
Mild case of LSD showing characteristic skin lesions (full body)



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FIGURE 6
Mild case of LSD showing characteristic skin lesions (neck)



©EEVA TUUPPAINEN

FIGURE 7
Severely affected cow with multiple skin lesions



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FIGURE 8
Severely affected cow with skin lesions covering the entire body, and enlarged lymph node



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FIGURE 9
Skin lesions in the perineum and genitalia



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FIGURE 10
Severe form of LSD with skin lesions in the head, neck, limbs and entire body



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FIGURE 11
Conjunctivitis and nodular skin lesions on the head



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FIGURE 12
Ulcerative lesions in the muzzle and lips



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FIGURE 13
Ulcerative skin lesion before scab formation



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FIGURE 14
Severe case of LSD with skin nodules covering the udder and teats



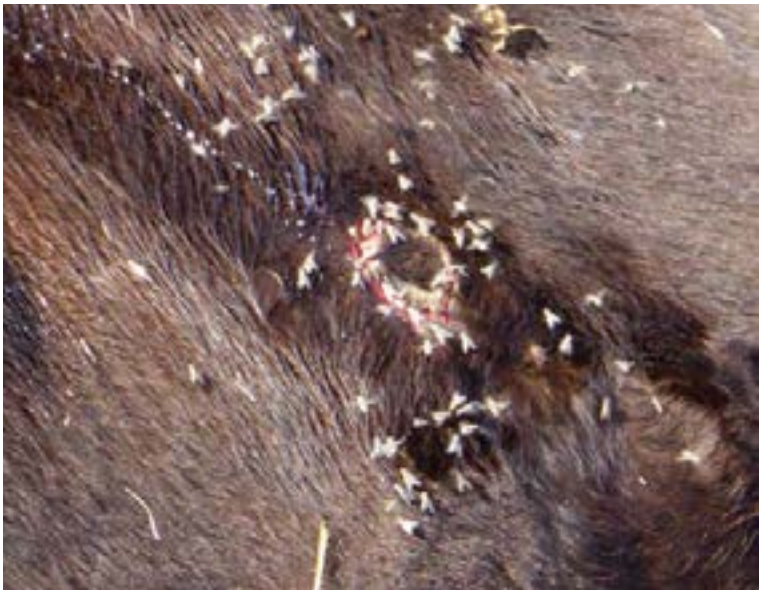
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FIGURE 15
Ulcerative lesion in the teat



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FIGURE 16
Skin lesion with a scab attracting domestic flies



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FIGURE 17
Skin lesions with scabs, ulcers and scars

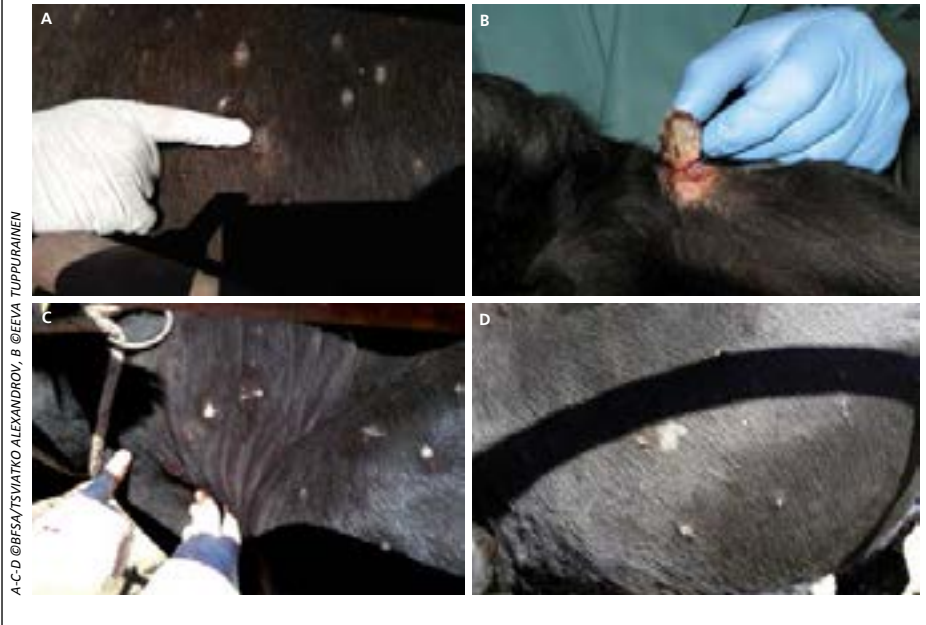
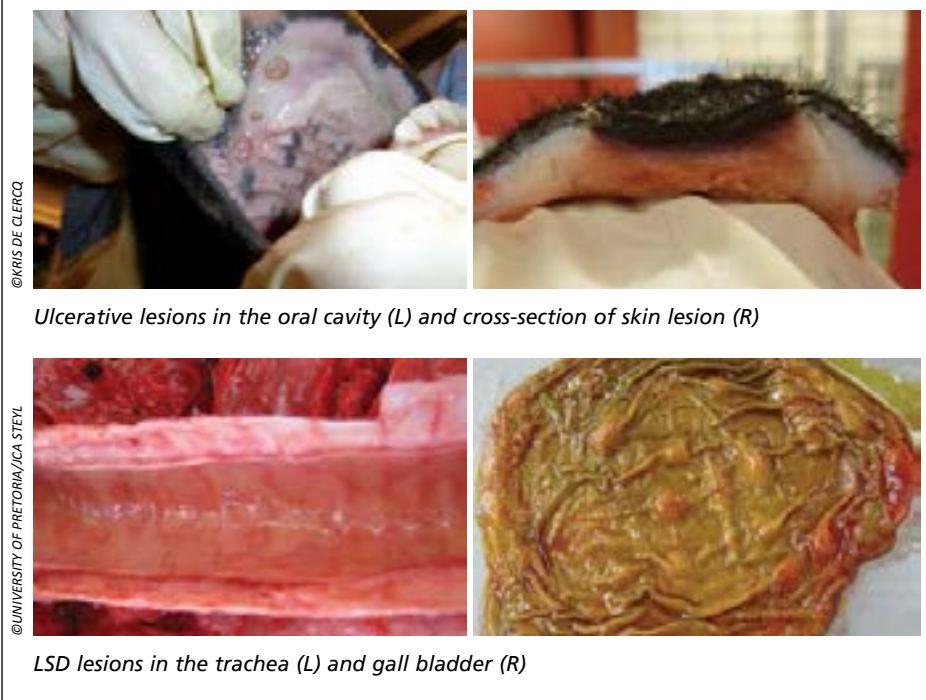


FIG 18
Internal LSD lesions



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) (Fig. 19): 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 (Fig. 20). The disease can be ruled out by detecting LSDV by PCR.
- Pseudocowpox (Parapoxvirus) (Fig. 21): lesions occur only on the teats and udder. The disease can be ruled out by detecting LSDV by PCR.
- Dermatophilosis (Fig. 22): early ringworm lesions, more superficial, clearly different, non-ulcerative surface structure of the ringworm lesion.
- Demodicosis (Fig. 23): dermal lesions predominantly over withers, neck, back, and flanks, often with alopecia present. The disease can be ruled out by detection of mites using skin scrapings.
- Bovine papular stomatitis (Parapoxvirus) (Fig. 24): lesions occur only in the mucous membranes of the mouth. The disease can be ruled out by PCR testing.
- Besnoitiosis (Fig. 25): lesions often occur in 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 (Fig. 26): dermal lesions most likely at ventral midline. The disease can be ruled out by PCR.

In addition, live, attenuated LSDV vaccines may cause mild adverse reactions in cattle which resemble clinical LSD (see pp. 37-40 for currently available vaccines).

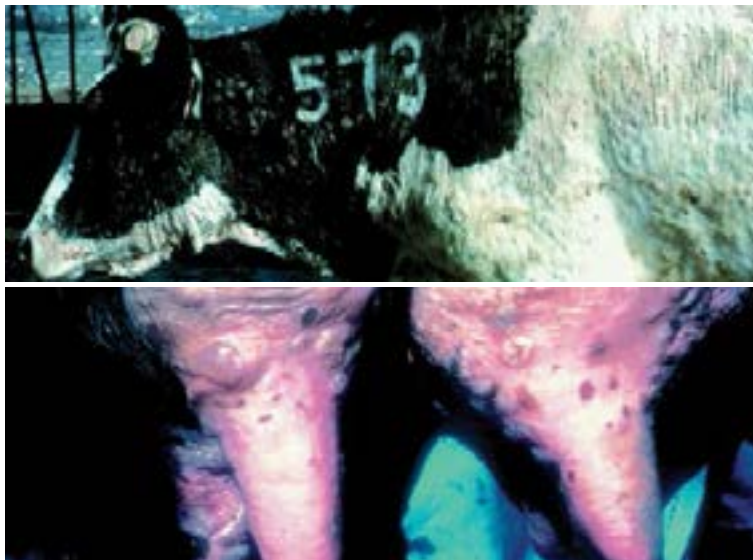
FIGURE 19
Bovine Herpesvirus 2



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Skin lesions covering the udder and teats

FIGURE 20
Pruriginous urticaria



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Urticaria covering most of the body (top) and teat vesicles (bottom) in dairy cattle, eight days after FMD vaccination.

FIGURE 21
Pseudocowpox lesions on the teats



©NOTTINGHAM SCHOOL OF VETERINARY MEDICINE

FIGURE 22
Ringworm



©BISA/TSVIATKO ALEXANDROV

Early ringworm lesions on the head (left) and neck (right)

FIGURE 23
Demodicosis skin lesions



©DOUW GROBLER

FIGURE 24
Papular stomatitis



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FIGURE 25
Besnoitiosis



©UNIVERSIDAD COMPLUTENSE DE MADRID/IGNACIO FERRE PÉREZ

FIGURE 26
Onchocerciasis lesions on the ventrum



©PROGRAMME ONCHOCERCOSIS, CAMEROON/ALFONS RENZ

Measures on the farm in case of suspicion

If a suspected case of LSD is detected by an owner, private veterinarian, animal trader, cattle truck driver, artificial inseminator, or any other visitor, a competent veterinary authority should be informed without delay and an official veterinarian/veterinary team should visit the farm to carry out an outbreak investigation.

Ideally, an investigation kit should be kept in each local veterinary office so that the attending veterinarian can set off to investigate with minimum delay. The equipment should include a digital camera, a GPS unit and a means of rapid communication (often a mobile phone, but could be a radio), as well as consumables and materials to collect and transport samples (FAO, *Good Emergency Management Practice* [GEMP], 2011). Measures at an affected farm holding should include:

- If possible, separate the suspected case(s) from the rest of the herd.
- Collect blood in EDTA tubes and whole blood for serum samples, saliva/nasal swabs and skin lesions or scabs for laboratory testing. In the case of several animals showing clinical signs, samples from approximately five animals should be sufficient for diagnosis. Detailed instructions for sample collection and transport are described in Section 7.
- Organize transport of the samples to a national reference laboratory without delay.
- Inform the competent authority and the reference laboratory that you are going to send samples containing potentially infectious LSD virus: indicate the number of samples you are sending.
- If possible, separate the rest of the animals from neighbouring herd(s) by feeding them on the farm and avoiding communal grazing.
- Neighbouring farmers, and those who have bought or sold animals from/to the affected farm recently, should be notified and placed under intensified surveillance. Cattle with or without clinical signs should be sampled.
- Stop cattle movement from/to the farm and limit visitors to essential services.
- Carry out clinical examinations on the rest of the animals (in each farm subunit) and systematically record the findings, including rectal temperature, in order to determine if any of the animals may already be incubating the disease. A prepared form may help you to record the findings efficiently. If a large number of animals are present, you may need to prioritize which animals you examine.
- Disinfect your hands, footwear, and outfit using any common disinfectant and when at home/office wash the clothes at +60 °C.
- Disinfect equipment and materials used in the affected holding, as well as the wheels of your vehicle on exit.

FIGURE 27
Clinical examination



Animal examination during an LSD outbreak in Georgia

- The application of spot-on repellents on the animals at the affected and neighbouring farms is highly recommended as a supportive measure to protect cattle from insects.
- If possible, transfer the rest of the day's veterinary farm visits to a colleague.

HOW TO CONDUCT AN OUTBREAK INVESTIGATION

Collecting, recording, and analysing epidemiological data on LSD outbreaks is crucial in order to implement an effective and feasible strategy to control and monitor the impact of activities. Carrying out an epidemiological interview requires specific skills in circumstances where farmers are likely to be under considerable stress. In an intensive cattle farming unit, the farm manager and workers often have more day-to-day contact with animals than the farm owner.

An outbreak investigation should prioritize the following:

- a) how long the disease has been present;
- b) magnitude of the problem: number of cases, definition of epidemiological units and population at risk;
- c) possible sources of infection;
- d) movements of animals, people, vehicles, or other fomites that may have spread the disease.

It is often helpful to sketch out a map of the area, showing the location of animal housing, animal groups, entry and exit points, and boundaries.

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.

Sample collection and shipping*

The sampling team should bring sufficient quantities of materials and equipment (see Box 1) for the number of animals to be sampled, plus a margin for materials that may be damaged or become unusable for other reasons (e.g. vacutainers that lose vacuum, etc.). Additionally, items for data collection, personal protection/biosecurity, and transport of samples must be packed. It is recommended to go with a field sampling form so that all needed samples and related information can be collected on-site. If submission of samples to a regional/international reference laboratory is foreseen, it is advisable to duplicate samples so that one set can be submitted while the other is safely stored.

Samples should be taken with care, using appropriate techniques, to avoid undue stress or injury to animals or harm to the sampler. Those in charge of sampling (and of conducting clinical inspections) should previously have been trained in techniques for restraining cattle (both for clinical inspection and sampling).

All samples that have not yet been tested should be considered infected and handled accordingly. All sampling materials used on farms should be disposed of safely and according to local regulations, e.g. bagged and transported back to the laboratory for autoclaving/appropriate disposal.

Diagnostic laboratories require the submission of suitable samples that are *clearly and permanently* labelled and that arrive at the laboratory in good condition.

PREFERRED SAMPLE TYPES

Skin lesions and scabs, saliva or nasal swabs, EDTA blood for PCR assay, whole blood for serum samples.

GENERAL RULES

Due to the highly characteristic clinical signs of LSD, it is not a common practice to carry out postmortem examinations in the field. Animals presenting a mild case of the disease do not usually have internal lesions, and there is no need to open severely diseased animals as their external lesions are so obvious. The indications listed below therefore refer to sampling from live animals.

- Use protective clothing.
- Restrain or sedate the animals in order to avoid stress or injury, and danger to the operators.
- Work aseptically, avoiding cross-contamination between samples; disinfect the sample collection site, change needles, scalpels, and gloves.
- Saliva and nasal swabs are collected using sterile swabs and placed into sterile tubes for transportation, with or without transport medium (Fig. 28).

* Adapted from Beltrán-Alcrudo *et al.*, 2017

BOX 1**Sampling materials*****General materials**

- labels and permanent markers;
- data collection forms, pens, clipboards;
- sharps bin for needle and scalpel disposal;
- (autoclavable) disposal bags.

Personal Protective Equipment**(PPE requirements will vary, e.g. surveillance vs. outbreak investigation)**

- dedicated clothing (coveralls)
- rubber boots
- boot covers
- gloves
- facemask
- (safety) glasses for eye protection
- disinfectant for hands
- disinfectant for boots.

Materials for sample transport

- primary containers/tubes/vials (leakproof and clearly labelled);
- absorbents;
- containers or bags capable of withstanding 95 kPa as secondary packaging, hermetically sealable (i.e. leakproof), preferably plastic, for storage of sample containers and blood tubes from each animal;
- cool box (+4 °C), either electric that can plug into a car (preferable) or other, e.g. Styrofoam box filled with cooling materials (ice, frozen water bottles or cold packs, as appropriate). Some commercially available eutectic cold packs with special gel allow the desired temperature to be kept for up to a couple of days. Portable, -80 °C freezers/dry shippers/liquid nitrogen tanks are only required if sampling takes place far from an appropriately equipped laboratory.

It is important always to maintain a "triple-layer" packing structure when transporting diagnostic samples.

Sampling materials for live animals

- materials for restraining animals;
- cotton wool and disinfectant to clean sampling site;
- sterile vacutainers (10 ml) without anti-coagulant (red stoppers) for serum collection;
- sterile vacutainers (10 ml) with EDTA (purple stoppers) for whole-blood collection;
- either vacutainer holders and vacutainer needles or 10-20 ml syringes. Different sizes of needles should be sufficient to avoid haemolysis;
- swabs;
- injectable local anaesthetic, disposable biopsy punches or scalpels, and suture material if full-thickness skin samples are to be collected from live animals.

Materials for postmortem sampling

- sample racks or cryoboxes for cryovials;
- 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.

* Adapted from Beltrán-Alcrudo *et al.*, 2017

FIGURE 28
Collection of saliva for PCR testing during an outbreak in Bulgaria



- 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.
- Scabs are excellent sample material as they are easy to collect, do not require sedation of the animal or local anaesthesia, survive long transport well in different temperatures and contain high concentrations of virus (Fig. 29).
- Collect blood samples from the jugular or tail vein (coccygeal vein).
- Collect sufficient volumes of blood: a minimum of 4 ml of vacutainer EDTA (purple top) is needed for PCR testing (note: heparin may hamper the PCR reaction) (Fig. 30). Tubes without an anticoagulant are used to collect serum samples. The tubes should be filled completely.
- After collection, tubes without anticoagulant should be allowed to stand at ambient temperature for at least 1-2 hours in an upright position to let the clot begin to contract. The clot can then be removed using a sterile rod and tubes stored at 4 °C for 12 hours. The serum can be removed with a pipette or decanted into fresh tubes. If it is necessary to clear the serum, the samples can be centrifuged at slow speed (1000 g/2000 rpm) for 15 minutes, after which serum can be removed. Paired serum samples can be collected 7-14 days apart.

FIGURE 29
Scabs are excellent sample material. Scab coming off leaves a raw ulcer



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FIGURE 30
Collection of blood samples from the tail vein in vacutainer EDTA for PCR testing



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TRANSPORT OF SAMPLES NATIONALLY AND INTERNATIONALLY

Diagnosing LSD is urgent and, to diagnose a disease correctly, it is essential that the right samples are selected, carefully labelled, packaged, and sent to the laboratory at the right temperature, using the fastest practical means of transport by the most direct route.

Samples must be accompanied by a submission form. The minimum amount of information required varies depending on the laboratory. It helps to phone the laboratory prior to sampling to ensure that submission procedures are followed correctly and the envisioned number of samples can be analysed or stored in good time.

In general, submission forms should contain the following information:

- number and type of samples and the source species;
- sample ID (one must be able to cross-reference each sample to the source animal);
- owner, name of farm, type of farming system;
- sampling location (address, county, district, province, country of origin, as appropriate);
- name of the person submitting the sample;
- name(s) of the person(s) to whom results are being sent;
- tests required;
- observed clinical signs, gross lesions;
- short epidemiological description: morbidity, mortality, number of affected animals, history, animals involved;
- potential differential diagnoses.

Triple packaging should be used even for road transport. Details of the characteristics of triple packaging can be found on pp. 30-31 – International Transport.

DISPATCH AND STORAGE OF SAMPLES

National transport

National regulations must be followed when transporting samples to the nearest laboratory, even if samples are transported by veterinary services staff.

Samples should reach the testing laboratory as soon as possible to prevent them from deteriorating and to ensure a reliable result, as well as to prevent the samples and the environment from being contaminated during transport. Shipped samples must be provided with adequate amounts of cooling materials, e.g. ice packs, to prevent deterioration.

Make sure to do the following:

- Fill sample submission form as described above.
- Mark/label the samples individually using a waterproof marker and, if labels are used, ensure that they will remain attached and are suitable for storage at -20-80 °C.
- Samples should be kept cool during transport to the laboratory by using a cool box with ice or freezer blocks.
- Send the samples in a leakproof, preferably triple container, with absorbent inside.

A. Blood, saliva swabs and tissue samples should be kept at 2-6 °C if the shipment is to last less than 48 hours and at -20 °C if it takes more than 48 hours.

B. Serum samples. If transport takes less than five days, the samples can be kept at 2-8 °C degrees in a refrigerator. If more than five days, the clot should be removed and samples stored at -20 °C degrees.

International transport

International transfer of infectious samples is usually expensive and time-consuming. Central veterinary authorities evaluate if samples need to be sent to an international reference laboratory for laboratory confirmation. If that is the case, the national reference laboratory is responsible for organizing the sample transport, usually with a courier specialized in the transfer of dangerous goods.

For Europe, the relevant regulation is the European Agreement on the International Carriage of Dangerous Goods by Road (ADR). For other regions, national regulations must be followed. If none are available, the UN Model Regulations set out in the 2016 OIE Manual for Diagnostic Tests and Vaccines for Terrestrial Animals (Sections 1.1.2 and 1.1.3) should be followed.

Potentially LSDV-infected samples are classified as Infectious Substances Class B (Division 6.2) and IATA packing instructions 650 must be followed (UN3373, Category B). It is forbidden to transport infectious substances as either carry-on baggage, checked baggage, or on one's person.

Prior to dispatch of samples, a contact at the reference laboratory must be informed of the shipment and shipment details must be agreed on. An import permit must be obtained from the reference laboratory and included with the sample transfer documents.

The receiving reference laboratory requires the following data:

- flight number/air waybill number;
- courier tracking number;
- date and time of expected arrival at the airport or the lab;
- two contact persons for potential queries, and details of those to whom test results should be sent (name, telephone number, fax number, e-mail address);
- completed sample submission form/cover letter.

The following documents must be enclosed with the sample package in a waterproof envelope, between the secondary and outer packaging, and also taped outside the package:

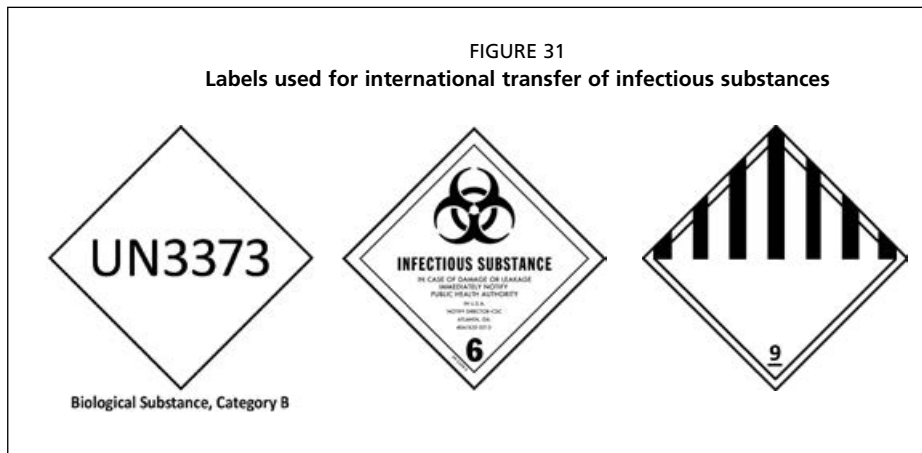
- import permit of the receiving laboratory;
- submission form/covering letter;
- a list of contents, including the sample type(s), numbers and volumes;
- air waybill;
- pro forma invoice – indicating that the samples are of no commercial value.

In most cases, use of dry ice is required to keep the samples frozen since transport, including customs procedures, usually takes more than five days.

Category B samples need to be transported inside triple containers. The primary (leak-proof, water resistant and sterile) container holds the sample. The lid of each sample container must be sealed with adhesive tape or parafilm, and wrapped with absorbent material. Several sealed, wrapped primary containers may be placed in one secondary container.

The secondary leakproof container should contain a sufficient amount of absorbent material. It is usually made out of plastic or metal and needs to meet IATA requirements. Dry ice cannot be placed inside the secondary container due to the risk of explosion.

Required labels must be affixed to the rigid outer (third) layer, with sufficient cushioning or dry ice inside. The following labels should be attached:



1. Infectious Substance/Hazard Label stating that the package contains a "Biological Substance, Category B" Animal Diagnostic Specimen of no Commercial Value (Hazard for Animal Health, not for people);
2. Full name, address and telephone number of sender;
3. Full name, address and telephone number of addressee;
4. Full name and telephone number of a responsible person knowledgeable about the shipment. RESPONSIBLE PERSON: First name LAST NAME, +123 4567 890;
5. Label reading "conserve at 4 °C" or "conserve at -70 °C", as appropriate;
6. Label for dry ice (if used) and the proper shipping name of the dry ice followed by the words "AS COOLANT". The net quantity of dry ice (in kilograms) must be clearly indicated;
7. UN number.

Laboratory confirmation of suspected cases and available diagnostic tools

VIRUS DETECTION

Basic diagnostic tests

National reference laboratories providing diagnostic services for LSD should participate in the annual inter-laboratory proficiency testing trials organized by the international reference laboratories or other appropriate institutes.

Several highly sensitive, well-validated, real-time and gel-based PCR methods are available and widely used to detect the presence of CaPV DNA, e.g. Bowden *et al.*, 2008; Stubbs *et al.*, 2012; Ireland & Binopal, 1998; Haegeman *et al.*, 2013; Tuppurainen *et al.*, 2005; Balinsky *et al.*, 2008.

These molecular assays cannot differentiate between LSDV, SPPV and GTPV, nor do they indicate whether or not the virus is still infectious. In general, performance of these tests is excellent. Electron microscopy examination can also be used for primary diagnostics although it is uncommon. Live virus can be isolated using various cell cultures of bovine or ovine origin.

Surveillance of an infectious virus in different matrixes is described by EFSA Scientific Opinion on lumpy skin disease (EFSA, 2015).

Differentiating a virulent from an attenuated LSDV strain

If characteristic clinical signs of LSD are detected in cattle vaccinated with vaccine containing attenuated LSDV, molecular assays are available to determine if the causative agent is the virulent field strain, or if the vaccine itself is causing an adverse reaction in vaccinated animals (Menasherow *et al.*, 2014; Menasherow *et al.*, 2016). Alternatively, sequencing of appropriate genes or gene fragments can be carried out (Gelaye *et al.*, 2015).

Differentiation between LSDV, SPPV and GTPV

Sometimes, clinical signs of LSD are detected in cattle vaccinated with vaccine containing attenuated SPPV or GTPV. In such cases a check should be made on whether the vaccine is offering protection or not, and whether the clinical signs are caused by the virulent field LSDV. Sometimes, although rarely, the SPP vaccine virus itself may cause adverse reactions.

Species-specific PCR methods can differentiate between LSDV, SPPV and GTPV (Lamien *et al.*, 2011a; Lamien *et al.*, 2011b; Le Goff *et al.*, 2009; Gelaye *et al.*, 2013).

Species-specific assays are also valuable tools if typical clinical signs of LSD are detected in wild ruminants in a country where all capripox members, LSD, SPP and GTP are endemic.

Recently, a method allowing the differentiation of eight pox viruses of medical and veterinary importance was published (Gelaye *et al.*, 2017). This can differentiate between LSDV, SPPV and GTPV, and also between LSD, bovine papular stomatitis, pseudocowpox and cowpox viruses.

DETECTION OF ANTIBODIES

In general, the immune status of a previously infected or vaccinated animal cannot be directly related to serum levels of neutralizing antibodies. Seronegative animals may have been infected at some point and antibody levels do not always rise in all vaccinated animals.

The levels of neutralizing antibodies start to rise approximately one week after detection of clinical signs, and affected animals reach the highest antibody levels approximately two to three weeks later. The antibody levels then begin to decrease, eventually dropping below detectable amounts.

During ongoing outbreaks, most of the infected animals seroconvert and serum samples can be tested using serum/virus neutralization, immunoperoxidase monolayer assay (IPMA) (Haegeman *et al.*, 2015) or indirect fluorescent antibody test (IFAT) (Gari *et al.*, 2008). It is highly likely that an LSD ELISA will also become commercially available soon.

During the inter-epizootic periods (i.e. the quiet periods/years between epidemics), serological surveillance is challenging because long-term immunity against LSDV is predominantly cell-mediated, and currently available serological tests may not be sufficiently sensitive to detect mild and long-standing LSDV infections.

ROLE OF THE NATIONAL REFERENCE LABORATORY

Rapid laboratory confirmation is essential in the successful control of an LSD outbreak. Thus, in all affected or at-risk countries, diagnostic capacity to carry out primary detection of LSDV should be in place so that control and eradication measures can be implemented without delay.

INTERNATIONAL REFERENCE LABORATORIES (CONTACT POINTS AND INFORMATION)

EU reference laboratory for LSD

CODA-CERVA, Belgium

Dr Annel De Vleeschauwer (annel.develeschauwer@coda-cerva.be)

Dr Kris De Clercq (kris.declercq@coda-cerva.be)

Groeselenberg 99

1180 Bruxelles

Belgium

Tel: +32 2 379 04 11 Fax: +32 2 379 04 01

E-mail: eurl-capripox@coda-cerva.be

OIE reference laboratories for LSD

Onderstepoort Veterinary Institute, South Africa

Agricultural Research Council

Dr David B. Wallace (WallaceD@arc.agric.za)

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Onderstepoort 0110

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Control and prevention of lumpy skin disease

For more information on available strategies, please consult the FAO Position on the sustainable prevention, control and elimination of LSD, particularly in Eastern Europe and the Balkans.

PREVENTION OF LUMPY SKIN DISEASE

- The best protection comes from prophylactic vaccination of the entire cattle population, carried out well in advance in at-risk areas.
- Movements of cattle inside the country and across borders should be strictly controlled or totally banned. Authorized cattle movements should be accompanied by a veterinary certificate including all data concerning the animals' origins, and animal health guarantees.
- In affected villages, cattle herds should be kept separate from other herds by avoiding communal grazing, if possible without animal welfare issues. However, in some cases the whole village forms a single epidemiological unit and then the feasibility of separation has to be evaluated on a case-by-case basis.
- Movements of vaccinated animals can be allowed within a restricted zone within a country after it has been established that full immunity has been provided by a vaccine with proven efficacy (28 days after vaccination).
- Cattle should be treated regularly with insect repellents to minimize the risk of vector transmission of the disease. This measure cannot fully prevent transmission but may reduce the risk.

CURRENTLY AVAILABLE VACCINES, SELECTION OF AN EFFECTIVE VACCINE, ADVERSE REACTIONS AND VACCINATION STRATEGY

Only live vaccines are currently available against LSDV. No Differentiation of Infected from Vaccinated Animals (DIVA) vaccines have been developed against LSD. Live vaccines are authorized for use in cattle in Africa, but in other currently affected regions specific authorization is required prior to their use.

Annual vaccination is recommended in affected countries, and harmonized vaccination campaigns across regions provide the best protection. Calves from naïve mothers should be vaccinated at any age, while calves from vaccinated or naturally infected mothers should be vaccinated at 3-6 months of age.

Regional harmonized vaccinations are recommended and should be carried out before large-scale movements of cattle, for example prior to the onset of seasonal grazing.

Live, attenuated LSDV vaccines may cause mild adverse reactions in cattle. Local reaction at the vaccination site (Fig. 32) is common and acceptable as it shows that the attenuated vaccine virus is replicating and producing good protection. Common adverse reactions

include temporary fever and a brief drop in milk yield. Some animals may show mild generalized disease. However, skin lesions caused by attenuated virus are usually superficial, clearly smaller, and different from those caused by the fully virulent field strain (Figs. 32-34). They disappear within 2-3 weeks without converting into necrotic scabs or ulcers.

In practice, vaccination campaigns are often started when the virus is already widespread in the region. Development of full protection from the vaccine takes approximately three weeks. During this time, cattle may still get infected by the field virus, and may show clinical signs despite being vaccinated. Some animals may also be incubating the virus when vaccinated, and in such cases clinical signs are detected less than ten days after vaccination.

Attenuated LSDV vaccines

Currently, there are three vaccine producers manufacturing attenuated LSDV vaccines. Live, attenuated LSDV vaccines provide good protection in cattle if 80 percent vaccination coverage is attained. In practice, all animals need to be vaccinated, including small calves and pregnant cows. Regional vaccination campaigns should be preferred to ring vaccination.

Attenuated SPPV vaccines

Sheep pox virus vaccines have been used in cattle against LSDV in those regions where LSD and SPP are both present. As the protection provided by SPPV vaccines against LSDV is believed to be partial, selection of the vaccine should always be based on demonstrated efficacy of the vaccine against LSDV by a challenge trial carried out in a controlled environment.

FIGURE 32
Local reaction at vaccination site



FIGURE 33
Post-vaccination superficial generalized skin lesions



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FIGURE 34
Post-vaccination superficial skin lesions in the udder



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If acceptable efficacy of the SPPV/GTPV vaccines is demonstrated, SPP vaccines can be used provided that full vaccination coverage and other appropriate control measures are in place.

Attenuated Gorgan GTPV vaccine

Commercially available GTPV Gorgan strain has been demonstrated to provide equal protection against LSD as the LSDV vaccines (Gari *et al.*, 2015). Gorgan GTPV vaccine is a good, cost-effective alternative in those countries where GTP and LSD overlap.

CATTLE MOVEMENT CONTROLS

Movements of unvaccinated cattle represent the major risk factor for disease spread. During an LSD outbreak, movements of cattle should be strictly regulated, but in practice effective control is often difficult. Appropriate legal powers should be in place to allow veterinary authorities to act as soon as any illegal transport of cattle is detected.

Trade in live cattle must be banned immediately upon suspicion and/or confirmation of the disease. In many regions, unauthorized transboundary trade occurs despite restrictions, underlining the importance of regional vaccination. Severe penalties should be applied for illegal movements.

Where nomadic and seasonal farming is practiced, cattle should be vaccinated at least 28 days before going on the move. Movements of unvaccinated breeding animals should not be allowed during outbreaks.

Slaughter of cattle should be allowed only in slaughterhouses located within restricted zones because open transport vehicles waiting at their destination may give blood-feeding, flying vectors sufficient time to transmit the virus.

STAMPING-OUT POLICIES AND DISPOSAL OF CARCASSES

In many affected countries, either total or partial stamping-out policies have been implemented. In countries with limited resources, no kind of stamping out may be affordable. The efficacy of these methods is widely discussed by experts and decision-makers. According to the EFSA urgent advice on lumpy skin disease, vaccination has a greater impact in reducing LSDV spread than any stamping-out policy (EFSA, 2016).

Stamping out should always be combined with a sound compensation programme. Without timely and adequate compensation, cattle owners are likely to object to having their animals killed, leading to reduced reporting and the dissemination of the disease through illegal movements of infected animals. The long-term effect of stamping out on farmers' livelihoods, public perception and media involvement should be considered in any decisions.

Total stamping out has the best chance of success and is practical if the first incursion of the disease in a country or defined region is detected and notified without delay, and the threat of repeated incursions is low.

Because identifying particularly mild and early cases may be extremely challenging, several weeks may elapse between initial infection and detection of the disease, allowing spread of the virus by vectors. In addition, the epidemiological unit involved may often be a whole village rather than a single farm, reducing the efficacy of total or partial stamping-out policies. Partial stamping out by culling animals with clinical disease may reduce infectivity, but is unlikely to end an outbreak on its own.

FIGURE 35
Burial of carcasses



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Timely, large-scale vaccination across the affected regions using an effective vaccine will bring an outbreak to a total halt regardless of the chosen stamping-out policy. However, the effect of the vaccination campaign may be felt earlier if total stamping out is undertaken.

When a stamping-out policy is implemented, culling and disposal of carcasses should take place as soon as possible in compliance with all animal welfare and safety requirements. Disposal by burial or burning should follow national rules on environmental protection. In some countries, these practices may not be allowed at all.

Appropriate methods for culling cattle are premedication and injection with barbiturates or other drugs, followed by captive-bolt stunning and pithing or free bullet. Disposal of carcasses should be conducted by burial, burning or rendering, according to national procedures.

Importantly, regardless of the stamping-out policy selected, severely affected animals should always be removed from the herd because they serve as a constant source of contamination for biting and blood-feeding vectors. In the same way, no animal showing any clinical signs of LSD should be sent to a slaughterhouse, but should be culled and disposed of either on-site or at an appropriate rendering plant. It should be borne in mind that farmers will benefit from replacement of culled animals with healthy, immunized ones as a herd usually needs several months to recover and is unlikely to return to the same level of production as before LSD infection.

CLEANING AND DISINFECTION OF PERSONNEL, PREMISES, AND THE ENVIRONMENT

Lumpy skin disease virus is very stable and survives well in extremely cold and dry environments within the pH range 6.3-8.3. Infected animals shed scabs from skin lesions. Inside the scabs, the virus may remain infectious for several months.

Thorough cleaning and disinfection with appropriate products should be performed on affected farms, trucks, premises and potentially contaminated environments. Personnel should also undergo disinfection.

Although LSDV is sensitive to most disinfectants and detergents, in order to effectively decontaminate animal facilities and holdings, mechanical removal of surface material such as dirt, manure, hay and straw is required beforehand. The disinfectant selected must be able to penetrate any organic material surrounding the infectious virus in the environment. FAO provides practical recommendations for decontamination of premises, equipment and the environment in the *Animal Health Manual on Procedures for Disease Eradication by Stamping Out* (FAO, 2001).

INSECT CONTROL ON ANIMALS AND IN THE ENVIRONMENT

Efficient insect control on cattle or in holdings may reduce the rate of mechanical transmission, but cannot totally prevent it, particularly where cattle are free-roaming or kept in fenced pastures. Anti-mosquito nets can be considered in cases when cattle are permanently kept indoors. The application of spot-on repellents can protect cattle from insects and ticks for short periods.

When insecticides are used, withdrawal times for milk and meat need to be considered. Large-scale use of insecticides in the environment is not recommended as it may be harmful to the ecological balance, and to other useful insects such as honeybees. Moreover, the risk to the environment is not fully understood.

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

FIGURE 36
Disinfection operations following an LSD outbreak



BIOSECURITY MEASURES AT HOLDINGS

In the event of LSD entering a country, farm biosecurity should be raised to the highest feasible level, taking into consideration the limits of the epidemiological unit in each case. As the disease is spread by vectors, such measures may not totally prevent an incursion, but the risk can be reduced.

Purchase of new animals that are either incubating the disease or are viraemic without exhibiting any symptoms presents a major risk of introducing the disease into a naïve herd. Introduction of new animals into herds should therefore be limited. Stock should be bought only from trusted sources. New animals should be examined and declared free of clinical signs prior to movement and on arrival, and should be kept separated/quarantined from the herd for at least 28 days.

Farm visits should be restricted to essential services with entry points to properties limited. All visitor vehicles and equipment should be cleaned in a wash-down bay when entering farms. Boots should also be cleaned or, alternatively, shoe covers should be worn. Visitors entering farms should wear clean protective clothing.

TARGET AUDIENCE FOR AWARENESS CAMPAIGNS

Awareness campaigns should be targeted at official and private veterinarians, both field and abattoir, veterinary students, farmers, herdsman, cattle traders, cattle truck drivers and artificial inseminators. Cattle truck drivers are in a particularly good position to identify infected animals on farms and in slaughterhouses and at cattle collection and resting stations, and to notify veterinary authorities of any clinical suspicion as soon as possible.

SURVEILLANCE PROGRAMMES

Surveillance programmes are based on active and passive clinical surveillance and laboratory testing of blood samples, nasal swabs, or skin biopsies collected from suspected cases.

As there are no DIVA vaccines against LSD, serological surveillance is of no use in affected countries or zones where the entire cattle population is vaccinated. However, serology can be used whenever the presence of unnoticed/unreported outbreaks are investigated in disease-free regions either bordering, or in close proximity to, affected regions with unvaccinated cattle. In such regions, the presence of seropositive animals can be considered as an indication of recent outbreaks.

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Lumpy skin disease (LSD) is a viral disease of cattle. Characterized by nodules on the skin, it is mostly transmitted by mosquitoes, by other hematophagous insects, and flies. The disease has dramatic effects on rural livelihoods, which are often strongly dependent on cattle, as it slashes milk production and may lead to sterility in bulls and fertility problems in females. It damages hides, and causes death due to secondary bacterial infections. Effects at national level are also devastating as the presence of the disease triggers strict trade restrictions.

Although traditionally limited to sub-Saharan Africa, LSD has slowly been invading new territories such as the Middle East and Turkey, and since 2015, most of the Balkan countries, the Caucasus and the Russian Federation, where the disease continues to spread. The risk of an imminent incursion into neighbouring, still unaffected countries, is very high.

In the current situation, veterinary services from affected and at-risk countries in the Middle East and Europe are facing the disease for first time. Official veterinarians, cattle farmers, and others along the value chain are therefore unfamiliar with LSD's clinical presentation, its transmission routes and the available prevention and control options. This manual aims to fill these gaps by providing veterinary professionals and paraprofessionals with the information they need to promptly diagnose and react to an outbreak of LSD. Cattle farmers will also benefit from reading it.

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