CHAPTER 3.4.12.

LUMPY SKIN DISEASE

SUMMARY

Description of the disease: *Lumpy skin disease (LSD) is a poxvirus disease of cattle characterised by fever, nodules on the skin, mucous membranes and internal organs, emaciation, enlarged lymph nodes, oedema of the skin, and sometimes death. The disease is of economic importance as it can cause a temporary reduction in milk production, temporary or permanent sterility in bulls, damage to hides and, occasionally, death. Various strains of capripoxvirus are responsible for the disease. These are antigenically indistinguishable from strains causing sheep pox and goat pox yet distinct at the genetic level. LSD has a partially different geographical distribution from sheep and goat pox, suggesting that cattle strains of capripoxvirus do not infect and transmit between sheep and goats. Transmission of LSD virus (LSDV) is thought to be predominantly by arthropods, natural contact transmission in the absence of vectors being inefficient. Lumpy skin disease is endemic in most African and Middle Eastern countries. Between 2012 and 2022, LSD spread into south-east Europe, the Balkans, Russia and Asia as part of the Eurasian LSD epidemic.*

Pathology: *the nodules are firm and may extend to the underlying subcutis and muscle. Acute histological key lesions consist of epidermal vacuolar changes with intracytoplasmic inclusion bodies and dermal vasculitis. Chronic key histological lesions consist of fibrosis and necrotic sequestrae.*

Detection of the agent: *Laboratory confirmation of LSD is most rapid using a real-time or conventional polymerase chain reaction (PCR) method specific for capripoxviruses in combination with a clinical history of a generalised nodular skin disease and enlarged superficial lymph nodes in cattle. Ultrastructurally, capripoxvirus virions are distinct from those of parapoxvirus, which causes bovine papular stomatitis and pseudocowpox, but cannot be distinguished morphologically from orthopoxvirus virions, including cowpox and vaccinia viruses, both of which can cause disease in cattle, although neither causes generalised infection and both are uncommon in cattle. LSDV will grow in tissue culture of bovine, ovine or caprine origin. In cell culture, LSDV causes a characteristic cytopathic effect and intracytoplasmic inclusion bodies that is distinct from infection with Bovine herpesvirus 2, which causes pseudo-lumpy skin disease and produces syncytia and intranuclear inclusion bodies in cell culture. Capripoxvirus antigens can be demonstrated in tissue culture using immunoperoxidase or immunofluorescent staining and the virus can be neutralised using specific antisera.*

A variety of conventional and real-time PCR tests as well as isothermal amplification tests using capripoxvirus-specific primers have been published for use on a variety of samples.

Serological tests: *The virus neutralisation test (VNT) and enzyme-linked immunosorbent assays (ELISAs) are widely used and have been validated. The agar gel immunodiffusion test and indirect immunofluorescent antibody test are less specific than the VNT due to cross-reactions with antibody to other poxviruses. Western blotting using the reaction between the P32 antigen of LSDV with test sera is both sensitive and specific, but is difficult and expensive to carry out.*

Requirements for vaccines: *All strains of capripoxvirus examined so far, whether derived from cattle, sheep or goats, are antigenically similar. Attenuated cattle strains, and strains derived from sheep and goats have been used as live vaccines against LSDV.*

A. INTRODUCTION

Lumpy skin disease (LSD) was first seen in Zambia in 1929, spreading into Botswana by 1943 (Haig, 1957), and then into South Africa the same year, where it affected over eight million cattle causing major economic loss. In 1957 it entered Kenya, associated with an outbreak of sheep pox (Weiss, 1968). In 1970 LSD spread north into the Sudan, by 1974 it had spread west as far as Nigeria, and in 1977 was reported from Mauritania, Mali, Ghana and Liberia. Another epizootic of LSD between 1981 and 1986 affected Tanzania, Kenya, Zimbabwe, Somalia and the Cameroon, with reported mortality rates in affected cattle of 20%. The occurrence of LSD north of the Sahara desert and outside the African continent was confirmed for the first time in Egypt and Israel between 1988 and 1989, and was reported again in 2006 (Brenner *et al.,* 2006). In the past decade, LSD occurrences have been reported in the Middle Eastern, European and Asian regions (for up-to-date information, consult WOAH WAHIS interface[1]). Lumpy skin disease outbreaks tend to be sporadic, depending upon animal movements, immune status, and wind and rainfall patterns affecting vector populations. The principal method of transmission is thought to be mechanical by various arthropod vectors (Tuppurainen *et al*., 2015).

Lumpy skin disease virus (LSDV) belongs to the family *Poxviridae*, subfamily *Chordopoxvirinae*, and genus *Capripoxvirus*. In common with other poxviruses LSDV replicates in the cytoplasm of an infected cell, forming distinct perinuclear viral factories. The LSD virion is large and brick-shaped measuring 293–299 nm (length) and 262–273 nm (width). The LSDV genome structure is also similar to other poxviruses, consisting of double-stranded linear DNA that is 25% GC-rich, approximately 150,000 bp in length, and encodes around 156 open reading frames (ORFs). An inverted terminal repeat sequence of 2200–2300 bp is found at each end of the linear genome. The linear ends of the genome are joined with a hairpin loop. The central region of the LSDV genome contains ORFs predicted to encode proteins required for virus replication and morphogenesis and exhibit a high degree of similarity with genomes of other mammalian poxviruses. The ORFs in the outer regions of the LSDV genome have lower similarity and likely encode proteins involved in viral virulence and host range determinants.

Phylogenetic analysis shows the majority of LSDV strains group into two monophyletic clusters (cluster 1.1 and 1.2) (Biswas *et al.,* 2020; Van Schalkwyk *et al.,* 2021). Cluster 1.1 consists of LSDV Neethling vaccine strains that are based on the LSDV/Neethling/LW-1959 vaccine strain (Kara *et al.,* 2003; Van Rooyen *et al.,* 1959; van Schalkwyk *et al.,* 2020) and historic wild-type strains from South Africa. Cluster 1.2 consists of wild-type strains from southern Africa, Kenya, the northern hemisphere, and the Kenyan KSGP O-240 commercial vaccine. In addition to these two clusters, there have recently been recombinant LSDV strains isolated from clinical cases of LSD in the field in Russia and central Asia (Flannery *et al.,* 2021; Sprygin *et al.,* 2018; 2020; Wang *et al.,* 2021). These recombinant viruses show unique patterns of accessory gene alleles, consisting of sections of both wild-type and "vaccine" LSDV strains.

The severity of the clinical signs of LSD is highly variable and depends on a number of factors, including the strain of capripoxvirus, the age of the host, immunological status and breed. *Bos taurus* is generally more susceptible to clinical disease than *Bos indicus;* the Asian buffalo (*Bubalus spp.*) has also been reported to be susceptible. Within *Bos taurus,* the fine-skinned Channel Island breeds develop more severe disease, with lactating cows appearing to be the most at risk. However, even among groups of cattle of the same breed kept together under the same conditions, there is a large variation in the clinical signs presented, ranging from subclinical infection to death (Carn & Kitching, 1995). There may be failure of the virus to infect the whole group, probably depending on the virulence of the virus isolate, immunological status of the host, host genotype and vector prevalence. Seroprevalence studies, experimental infections and case reports have provided indications that several wildlife species (e.g. springbok, impala, giraffe, camel, banteng) are susceptible to LSDV infection (Dao *et al.,* 2022; Hedger & Hamblin, 1983; Kumar *et al.,* 2023; Porco *et al.,* 2023). The scarcity of documented outbreaks in wildlife and the fact that available studies remain limited in number and mostly involve only a few animals, make it difficult to determine the role of wildlife in LSDV epidemiology. This topic deserves further study, especially given the current spread of LSDV in new geographical areas where large numbers of naïve, potentially susceptible wild bovines and other ruminants are present.

The incubation period under field conditions has not been reported, but following experimental inoculation is 6–9 days until the onset of fever. In the acutely infected animal, there is an initial pyrexia, which may exceed 41°C and persist for 1 week. All the superficial lymph nodes become enlarged. In lactating cattle there is a marked reduction in milk yield. Lesions develop over the body, particularly on the head, neck, udder, scrotum, vulva and perineum between 7 and 19 days after virus inoculation (Coetzer, 2004). The characteristic integumentary lesions are multiple, well circumscribed to coalescing, 0.5–5 cm in diameter, firm, flat-topped papules and nodules. The nodules involve the dermis and epidermis, and may extend to the underlying subcutis and occasionally to the adjacent striated muscle. These nodules have a creamy grey to white colour on cut section, which may initially

1 https://www.woah.org/en/what-we-do/animal-health-and-welfare/disease-data-collection/

exude serum, but over the ensuing 2 weeks a cone-shaped central core or sequestrum of necrotic material/necrotic plug ("sit-fast") may appear within the nodule. The acute histological lesions consist of epidermal vacuolar changes with intracytoplasmic inclusion bodies and dermal vasculitis. The inclusion bodies are numerous, intracytoplasmic, eosinophilic, homogenous to occasionally granular and they may occur in endothelial cells, fibroblasts, macrophages, pericytes, and keratinocytes. The dermal lesions include vasculitis with fibrinoid necrosis, oedema, thrombosis, lymphangitis, dermal-epidermal separation, and mixed inflammatory infiltrate. The chronic lesions are characterised by an infarcted tissue with a sequestered necrotic core, often rimmed by granulation tissue gradually replaced by mature fibrosis. At the appearance of the nodules, the discharge from the eyes and nose becomes mucopurulent, and keratitis may develop. Nodules may also develop in the mucous membranes of the mouth and alimentary tract, particularly the abomasum and in the trachea and the lungs, resulting in primary and secondary pneumonia. The nodules on the mucous membranes of the eyes, nose, mouth, rectum, udder and genitalia quickly ulcerate, and by then all secretions, ocular and nasal discharge and saliva contain LSD virus (LSDV). The limbs may be oedematous and the animal is reluctant to move. Pregnant cattle may abort, and there is a report of intrauterine transmission (Rouby & Aboulsoudb, 2016). Bulls may become permanently or temporarily infertile and the virus can be excreted in the semen for prolonged periods (Irons *et al*., 2005). Recovery from severe infection is slow; the animal is emaciated, may have pneumonia and mastitis, and the necrotic plugs of skin, which may have been subject to fly strike, are shed leaving deep holes in the hide (Prozesky & Barnard, 1982).

The main differential diagnosis is pseudo-LSD caused by bovine herpesvirus 2 (BoHV-2). This is usually a milder clinical condition, characterised by superficial nodules, resembling only the early stage of LSD. Intra-nuclear inclusion bodies and viral syncytia are histopathological characteristics of BoHV-2 infection not seen in LSD. Other differential diagnoses (for integumentary lesions) include: dermatophilosis, dermatophytosis, bovine farcy, photosensitisation, actinomycosis, actinobacillosis, urticaria, insect bites, besnoitiosis, nocardiosis, demodicosis, onchocerciasis, pseudo-cowpox, and cowpox. Differential diagnoses for mucosal lesions include: foot and mouth disease, bluetongue, mucosal disease, malignant catarrhal fever, infectious bovine rhinotracheitis, and bovine papular stomatitis.

LSDV is not transmissible to humans. However, all laboratory manipulations must be performed at an appropriate containment level determined using biorisk analysis (see Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*).

B. DIAGNOSTIC TECHNIQUES

Table 1. Test methods available for the diagnosis of LSD and their purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent						
Virus isolation	+	++	+	+++	+	–
PCR	++	+++	++	+++	+	–
TEM	–	–	–	+	–	–
Detection of immune response						
VNT	++	++	++	++	++	++
IFAT	+	+	+	+	+	+
ELISA	++	++	++	++	++	++

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
PCR = polymerase chain reaction; TEM = Transmission electron microscopy; VNT = virus neutralisation test;
IFAT = indirect fluorescent antibody test; ELISA = enzyme-linked immunosorbent assay.

1. Detection of the agent

1.1. Specimen collection, submission and preparation

Material for virus isolation and antigen detection should be collected as biopsies or from skin nodules at post-mortem examination. Samples for virus isolation should preferably be collected within the first week of the occurrence of clinical signs, before the development of neutralising antibodies (Davies, 1991; Davies *et al*., 1971), however virus can be isolated from skin nodules for at least 3–4 weeks thereafter. Samples for genome detection using conventional or real-time polymerase chain reaction (PCR) may be collected when neutralising antibody is present. Following the first appearance of the skin lesions, the virus can be isolated for up to 35 days and viral nucleic acid can be demonstrated via PCR for up to 3 months (Tuppurainen *et al*., 2005; Weiss, 1968). Buffy coat from blood collected into heparin or EDTA (ethylene diamine tetra-acetic acid) during the viraemic stage of LSD (before generalisation of lesions or within 4 days of generalisation), can also be used for virus isolation. Samples for histology should include the lesion and tissue from the surrounding (non-lesion) area, be a maximum size of 2 cm^3, and be placed immediately following collection into ten times the sample volume of 10% neutral buffered formal saline.

Tissues in formalin have no special transportation requirements in regard to biorisks. Blood samples with anticoagulant for virus isolation from the buffy coat should be placed immediately on ice after gentle mixing and processed as soon as possible. In practice, the samples may be kept at 4°C for up to 2 days prior to processing, but should not be frozen or kept at ambient temperatures. Tissues for virus isolation and antigen detection should be kept at 4°C, on ice or at –20°C. If it is necessary to transport samples over long distances without refrigeration, the medium should contain 10% glycerol; the samples should be of sufficient size (e.g. 1 g in 10 ml) that the transport medium does not penetrate the central part of the biopsy, which should be used for virus isolation.

Material for histology should be prepared using standard techniques and stained with haematoxylin and eosin (H&E) (Burdin, 1959). Lesion material for virus isolation and antigen detection is minced using a sterile scalpel blade and forceps and then macerated in a sterile steel ball-bearing mixer mill, or ground with a pestle in a sterile mortar with sterile sand and an equal volume of sterile phosphate buffered saline (PBS) or serum-free modified Eagle's medium containing sodium penicillin (1000 international units [IU]/ml), streptomycin sulphate (1 mg/ml), mycostatin (100 IU/ml) or fungizone (amphotericin, 2.5 μg/ml) and neomycin (200 IU/ml). The suspension is freeze–thawed three times and then partially clarified using a bench centrifuge at 600 ***g*** for 10 minutes. In cases where bacterial contamination of the sample is expected (such as when virus is isolated from skin samples), the supernatant can be filtered through a 0.45 μm pore size filter after the centrifugation step. Buffy coats may be prepared from unclotted blood using centrifugation at 600 ***g*** for 15 minutes, and the buffy coat carefully removed into 5 ml of cold double-distilled water using a sterile Pasteur pipette. After 30 seconds, 5 ml of cold double-strength growth medium is added and mixed. The mixture is centrifuged at 600 ***g*** for 15 minutes, the supernatant is discarded and the cell pellet is suspended in 5 ml growth medium, such as Glasgow's modified Eagle's medium (GMEM). After centrifugation at 600 ***g*** for a further 15 minutes, the resulting pellet is suspended in 5 ml of fresh GMEM. Alternatively, the buffy coat may be separated from a heparinised sample by using a Ficoll gradient.

1.2. Virus isolation on cell culture

LSDV will grow in tissue culture of bovine, ovine or caprine origin. MDBK (Madin–Darby bovine kidney) cells are often used, as they support good growth of the virus and are well characterised (Fay *et al.,* 2020). Primary cells, such as lamb testis (LT) cells also support viral growth, but care needs to be taken to ensure they are not contaminated with viruses such as bovine viral diarrhoea virus. One ml of clarified supernatant or buffy coat is inoculated onto a confluent monolayer in a 25 cm^2 culture flask at 37°C and allowed to adsorb for 1 hour. The culture is then washed with warm PBS and covered with 10 ml of a suitable medium, such as GMEM, containing antibiotics and 2% fetal calf serum. If available, tissue culture tubes containing appropriate cells and a flying cover-slip, or tissue culture microscope slides, are also infected.

The flasks/tissue culture tubes are examined daily for 7–14 days for evidence of cytopathic effects (CPE). Infected cells develop a characteristic CPE consisting of retraction of the cell membrane from surrounding cells, and eventually rounding of cells and margination of the nuclear chromatin. At first only small areas of CPE can be seen, sometimes as soon as 2 days after infection; over the following 4–6 days

these expand to involve the whole cell monolayer. If no CPE is apparent by day 14, the culture should be freeze–thawed three times, and clarified supernatant inoculated on to a fresh cell monolayer. At the first sign of CPE in the flasks, or earlier if a number of infected cover-slips are being used, a cover-slip should be removed, fixed in acetone and stained using H&E. Eosinophilic intracytoplasmic inclusion bodies, which are variable in size but up to half the size of the nucleus and surrounded by a clear halo, are diagnostic for poxvirus infection. PCR may be used as an alternative to H&E for confirmation of the diagnosis. The CPE can be prevented or delayed by adding specific anti-LSDV serum to the medium. In contrast, the herpesvirus that causes pseudo-LSD produces a Cowdry type A intranuclear inclusion body. It also forms syncytia.

An ovine testis cell line (OA3.T) has been evaluated for the propagation of capripoxvirus isolates (Babiuk *et al.*, 2007), however this cell line has been found to be contaminated with pestivirus and should be used with caution.

1.3. Polymerase chain reaction (PCR)

The conventional gel-based PCR method described below is a simple, fast and sensitive method for the detection of capripoxvirus genome in EDTA blood, semen or tissue culture samples (Tuppurainen *et al*., 2005).

1.3.1. Test procedure

The extraction method described below can be replaced using commercially available DNA extraction kits.

i) Freeze and thaw 200 µl of blood in EDTA, semen or tissue culture supernatant and suspend in 100 µl of lysis buffer containing 5 M guanidine thiocyanate, 50 mM potassium chloride, 10 mM Tris/HCl (pH 8); and 0.5 ml Tween 20.

ii) Cut skin and other tissue samples into fine pieces using a sterile scalpel blade and forceps. Grind with a pestle in a mortar. Suspend the tissue samples in 800 µl of the above mentioned lysis buffer.

iii) Add 2 µl of proteinase K (20 mg/ml) to blood samples and 10 µl of proteinase K (20 mg/ml) to tissue samples. Incubate at 56°C for 2 hours or overnight, followed by heating at 100°C for 10 minutes. Add phenol:chloroform:isoamylalcohol (25:24:1 [v/v]) to the samples in a 1:1 ratio. Vortex and incubate at room temperature for 10 minutes. Centrifuge the samples at 16,060 ***g*** for 15 minutes at 4°C. Carefully collect the upper, aqueous phase (up to 200 µl) and transfer into a clean 2.0 ml tube. Add two volumes of ice cold ethanol (100%) and 1/10 volume of 3 M sodium acetate (pH 5.3). Place the samples at –20°C for 1 hour. Centrifuge again at 16,060 ***g*** for 15 minutes at 4°C and discard the supernatant. Wash the pellets with ice cold 70% ethanol (100 µl) and centrifuge at 16,060 ***g*** for 1 minute at 4°C. Discard the supernatant and dry the pellets thoroughly. Suspend the pellets in 30 µl of nuclease-free water and store immediately at –20°C (Tuppurainen *et al*., 2005). Alternatively, a column-based extraction kit may be used.

iv) The primers for this PCR assay were developed from the gene encoding the viral attachment protein. The size of the expected amplicon is 192 bp (Ireland & Binepal, 1998). The primers have the following gene sequences:

Forward primer 5'-TCC-GAG-CTC-TTT-CCT-GAT-TTT-TCT-TAC-TAT-3'

Reverse primer 5'-TAT-GGT-ACC-TAA-ATT-ATA-TAC-GTA-AAT-AAC-3'.

v) DNA amplification is carried out in a final volume of 50 µl containing: 5 µl of 10 × PCR buffer, 1.5 µl of $MgCl_2$ (50 mM), 1 µl of dNTP (10 mM), 1 µl of forward primer, 1 µl of reverse primer, 1 µl of DNA template (~10 ng), 0.5 µl of *Taq* DNA polymerase and 39 µl of nuclease-free water. The volume of DNA template required may vary and the volume of nuclease-free water must be adjusted to the final volume of 50 µl.

vi) Run the samples in a thermal cycler as follows: 2 minutes at 95°C; then 45 seconds at 95°C, 50 seconds at 50°C and 1 minute at 72°C (34 cycles); 2 minutes at 72°C and hold at 4°C until analysis.

vii) Mix 10 µl of each sample with loading dye and load onto a 1.5% agarose gel in TAE buffer (Tris/acetate buffer containing EDTA). Load a parallel lane with a 100 bp DNA-marker ladder. Electrophoretically separate the products using approximately 8–10 V/cm for 40–60 minutes and visualise with a suitable DNA stain and transilluminator.

Quantitative real-time PCR methods have been described that are reported to be faster and have higher sensitivity than conventional PCRs (Balinsky *et al*., 2008; Bowden *et al*., 2008). A real-time PCR method that differentiates between LSDV, sheep pox virus and goat pox virus has been published (Lamien *et al.,* 2011).

Quantitative real-time PCR assays have been designed to differentiate between Neethling-based LSDV strains, which are often used for vaccination, and wild-type LSDV strains from cluster 1.2 (Agianniotaki *et al.,* 2017; Pestova *et al.,* 2018; Vidanovic *et al.,* 2016). These "DIVA" assays (DIVA: differentiation of infected from vaccinated animals) enable, for example, differentiation of "Neethling response" caused by vaccination with a LSDV Neethling vaccine strain from disease caused by infection with a cluster 1.2 wild-type virus. However these DIVA PCR assays cannot distinguish between a LSDV Neethling vaccine strain and the novel recombinant LSDV strains isolated from disease outbreaks in Asia (Flannery *et al.,* 2021). These DIVA assays are also not capable of discriminating between LSDV Neethling vaccine strains and recently characterised (historic) wild-type viruses from South Africa belonging within cluster 1.1 (Van Schalkwyk *et al.,* 2020; 2021). Consequently, in regions where recombinant strains (currently Asia and possibly elsewhere) or wild-type cluster 1.1 strains are circulating (currently South Africa and possibly elsewhere), these DIVA assays are not suitable for distinguishing vaccine and wild-type virus. Thus, in order to overcome these constraints, whole genome sequencing is recommended.

1.4. Transmission electron microscopy

The characteristic poxvirus virion can be visualised using a negative staining preparation technique followed by examination with an electron microscope. There are many different negative staining protocols, an example of which is given below.

1.4.1. Test procedure

Before centrifugation, material from the original biopsy suspension is prepared for examination under the transmission electron microscope by floating a 400-mesh hexagonal electron microscope grid, with pioloform-carbon substrate activated by glow discharge in pentylamine vapour, onto a drop of the suspension placed on parafilm or a wax plate. After 1 minute, the grid is transferred to a drop of Tris/EDTA buffer, pH 7.8, for 20 seconds and then to a drop of 1% phosphotungstic acid, pH 7.2, for 10 seconds. The grid is drained using filter paper, air-dried and placed in the electron microscope. The capripox virion is brick shaped, covered in short tubular elements and measures approximately 290 × 270 nm. A host-cell-derived membrane may surround some of the virions, and as many as possible should be examined to confirm their appearance (Kitching & Smale, 1986).

The virions of capripoxvirus are indistinguishable from those of orthopoxvirus, but, apart from vaccinia virus and cowpox virus, which are both uncommon in cattle and do not cause generalised infection, no other orthopoxvirus is known to cause lesions in cattle. However, vaccinia virus may cause generalised infection in young immunocompromised calves. In contrast, orthopoxviruses are a common cause of skin disease in domestic buffalo (*Bubalus bubalis*) causing buffalo pox, a disease that usually manifests as pock lesions on the teats, but may cause skin lesions at other sites, such as the perineum, the medial aspects of the thighs and the head. Orthopoxviruses that cause buffalo pox cannot be readily distinguished from capripoxvirus by electron microscopy. The virions of parapoxvirus that cause bovine papular stomatitis and pseudocowpox are smaller, oval in shape and each is covered in a single continuous tubular element that appears as striations over the virion. Capripoxvirus virions are also distinct from the herpesvirus that causes pseudo-LSD (also known as "Allerton" or bovine herpes mammillitis).

1.5. Fluorescent antibody tests

Capripoxvirus antigen can be identified on infected cover-slips or tissue culture slides using fluorescent antibody tests. Cover-slips or slides should be washed and air-dried and fixed in cold acetone for

10 minutes. The indirect test using immune cattle sera is subject to high background colour and nonspecific reactions. However, a direct conjugate can be prepared from sera from convalescent cattle (or from sheep or goats convalescing from capripox) or from rabbits hyperimmunised with purified capripoxvirus. Uninfected tissue culture should be included as a negative control as cross-reactions can cause problems due to antibodies to cellular components (pre-absorption of these from the immune serum helps solve this issue).

1.6. Immunohistochemistry

Immunohistochemistry using F80G5 monoclonal antibody specific for capripoxvirus ORF 057 has been described for detection of LSDV antigen in the skin of experimentally infected cattle (Babiuk *et al.,* 2008).

1.7. Isothermal genome amplification

Molecular tests using loop-mediated isothermal amplification to detect capripoxvirus genomes are reported to provide sensitivity and specificity similar to real-time PCR with a simpler method and lower cost (Das *et al.,* 2012; Murray *et al.,* 2013). Field validation of the Das *et al.* method was reported by Omoga *et al.* (2016).

2. Serological tests

All the viruses in the genus *Capripoxvirus* share a common major antigen for neutralising antibodies and it is thus not possible to distinguish strains of capripoxvirus from cattle, sheep or goats using serological techniques.

2.1. Virus neutralisation

A test serum can either be titrated against a constant titre of capripoxvirus (100 $TCID_{50}$ [50% tissue culture infective dose]) or a standard virus strain can be titrated against a constant dilution of test serum in order to calculate a neutralisation index. Because of the variable sensitivity of tissue culture to capripoxvirus, and the consequent difficulty of ensuring the accurate and repeatable seeding of 100 $TCID_{50}$/well, the neutralisation index is the preferred method in most laboratories, although it does require a larger volume of test sera. The test is described using 96-well flat-bottomed tissue-culture grade microtitre plates, but it can be performed equally well in tissue culture tubes with the appropriate changes to the volumes used, although it is more difficult to read an end-point in tubes.

2.1.1. Test procedure

i) Test sera, including a negative and a positive control, are diluted 1/5 in Eagle's/HEPES (N-2-hydroxyethylpiperazine, N-2-ethanesulphonic acid) buffer and inactivated at 56°C for 30 minutes.

ii) Next, 50 µl of the first inactivated serum is added to columns 1 and 2, rows A to H of the microtitre plate. The second serum is placed in columns 3 and 4, the third in columns 5 and 6, the positive control serum is placed in columns 7 and 8, the negative control serum is placed in columns 9 and 10, and 50 µl of Eagle's/HEPES buffer (without serum) is placed in columns 11 and 12, and to all wells in row H.

iii) A reference strain of capripoxvirus, usually a vaccine strain known to grow well in tissue culture, with a titre of over $\log_{10}$ 6 $TCID_{50}$ per ml is diluted in Eagle's/HEPES in bijoux bottles to give a log dilution series of $\log_{10}$ 5.0, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5 $TCID_{50}$ per ml (equivalent to $\log_{10}$ 3.7, 2.7, 2.2, 1.7, 1.2, 0.7, 0.2 $TCID_{50}$ per 50 µl).

iv) Starting with row G and the most diluted virus preparation, 50 µl of virus is added to each well in that row. This is repeated with each virus dilution, the highest titre virus dilution being placed in row A.

v) The plates are covered and incubated for 1 hour at 37°C.

vi) An appropriate cell suspension (such as MDBK cells) is prepared from pregrown monolayers as a suspension of 10^5 cells/ml in Eagle's medium containing antibiotics and 2% fetal calf serum. Following incubation of the microtitre plates, 100 µl of cell suspension is

added to all the wells, except wells H11 and H12, which serve as control wells for the medium. The remaining wells of row H are cell and serum controls.

vii) The microtitre plates are covered and incubated at 37°C for 9 days.

viii) Using an inverted microscope, the monolayers are examined daily from day 4 for evidence of CPE. There should be no CPE in the cells of row H. Using the 0240 KSGP vaccine strain of capripoxvirus, by way of example, the final reading is taken on day 9, and the titre of virus in each duplicate titration is calculated using the Kärber method. If left longer, there is invariably a 'breakthrough' of virus in which virus that was at first neutralised appears to disassociate from the antibody.

ix) *Interpretation of the results:* The neutralisation index is the log titre difference between the titre of the virus in the negative serum and in the test serum. An index of ≥1.5 is positive. The test can be made more sensitive if serum from the same animal is examined before and after infection. Because the immunity to capripoxviruses is predominantly cell mediated, a negative result, particularly following vaccination, after which the antibody response may be low, does not imply that the animal from which the serum was taken is not protected.

Antibodies to capripoxvirus can be detected from 1 to 2 days after the onset of clinical signs. These remain detectable for about 7 months.

2.2. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assays (ELISAs) for the detection of capripoxviral antibodies are widely used and are available in commercial kit form (Milovanovic *et al.,* 2019; Samojlovic *et al.,* 2019).

2.3. Indirect fluorescent antibody test

Capripoxvirus-infected tissue culture grown on cover-slips or tissue culture microscope slides can be used for the indirect fluorescent antibody test. Uninfected tissue culture control, and positive and negative control sera, should be included in the test. The infected and control cultures are fixed in acetone at –20°C for 10 minutes and stored at 4°C. Dilutions of test sera are made in PBS, starting at 1/20 or 1/40, and positive samples are identified using an anti-bovine gamma-globulin conjugated with fluorescein isothiocyanate. Antibody titres may exceed 1/1000 after infection. Sera may be screened at 1/50 and 1/500. Cross-reactions can occur with orf virus (contagious pustular dermatitis virus of sheep), bovine papular stomatitis virus and perhaps other poxviruses.

2.4. Western blot analysis

Western blotting of test sera against capripoxvirus-infected cell lysate provides a sensitive and specific system for the detection of antibody to capripoxvirus structural proteins, although the test is expensive and difficult to carry out.

Capripoxvirus-infected LT cells should be harvested when 90% CPE is observed, freeze–thawed three times, and the cellular debris pelleted using centrifugation. The supernatant should be decanted, and the proteins should be separated using SDS/PAGE (sodium dodecyl sulphate/polyacrylamide gel electrophoresis). A vertical discontinuous gel system, using a stacking gel made up of acrylamide (5%) in Tris (125 mM), pH 6.8, and SDS (0.1%), and a resolving gel made up of acrylamide (10–12.5%) in Tris (560 mM), pH 8.7, and SDS (0.1%), is recommended for use with a glycine running buffer containing Tris (250 mM), glycine (2 M), and SDS (0.1%). Samples of supernatant should be prepared by boiling for 5 minutes with an appropriate lysis buffer prior to loading. Alternatively, purified virus or recombinant antigens may replace tissue-culture-derived antigen.

Molecular weight markers should be run concurrently with the protein samples. The separated proteins in the SDS/PAGE gel should be transferred electrophoretically to a nitrocellulose membrane (NCM). After transfer, the NCM is rinsed thoroughly in PBS and blocked in 3% bovine serum albumin (BSA) in PBS, or 5% skimmed milk powder in PBS, on a rotating shaker at 4°C overnight. The NCM can then be separated into strips by employing a commercial apparatus to allow the concurrent testing of multiple serum samples, or may be cut into strips and each strip incubated separately thereafter. The NCM is washed thoroughly with five changes of PBS for 5 minutes on a rotating shaker, and then incubated at room temperature on the shaker for 1.5 hours, with the appropriate serum at a dilution of 1/50 in blocking

buffer (3% BSA and 0.05% Tween 20 in PBS; or 5% milk powder and 0.05% Tween 20 in PBS). The membrane is again thoroughly washed and incubated (in blocking buffer) with anti-species immunoglobulin horseradish-peroxidase-conjugated immunoglobulins at a dilution determined using titration. After further incubation at room temperature for 1.5 hours, the membrane is washed and a solution of diaminobenzidine tetrahydrochloride (10 mg in 50 ml of 50 mM Tris/HCl, pH 7.5, and 20 µl of 30% [v/v] hydrogen peroxide) is added. Incubation is then undertaken for approximately 3–7 minutes at room temperature on a shaker with constant observation, and the reaction is stopped by washing the NCM in PBS before excessive background colour is seen. A positive and negative control serum should be used on each occasion.

Positive test samples and the positive control will produce a pattern consistent with reaction to proteins of molecular weights 67, 32, 26, 19 and 17 kDa – the major structural proteins of capripoxvirus – whereas negative serum samples will not react with all these proteins. Hyperimmune serum prepared against parapoxvirus (bovine papular stomatitis or pseudocowpox virus) will react with some of the capripoxvirus proteins, but not the 32 kDa protein that is specific for capripoxvirus.

C. REQUIREMENTS FOR VACCINES

1. Background: rationale and intended use of the product

Vaccines are a key tool to control LSD. Different types of LSD vaccines have been developed and several are commercially available (Tuppurainen *et al.,* 2021).

Live attenuated vaccines (LAV) based on the Neethling LSDV strain (homologous LAV vaccines) have been shown to offer high levels of protection against LSD under experimental conditions (Haegeman *et al.,* 2021) and have been used successfully to control the disease in the field, through systematic vaccination of the entire country's cattle population for a number of consecutive years (Klement *et al.,* 2020). Homologous vaccines may induce fever, produce a local reaction at the site of inoculation, cause a temporary reduction in milk production and on rare occasions induce a 'Neethling' response (Ben-Gera *et al.,* 2015; Davies, 1991; Haegeman *et al.,* 2021). Such adverse effects, however, usually resolve within a few days and are largely outweighed by the overall benefits of vaccination with homologous vaccines. The duration of immunity induced by good quality live attenuated LSDV vaccines was shown to be at least 18 months (Haegeman *et al.,* 2023).

As capripoxviruses provide cross-reactive protection within the genus, heterologous LAVs comprising sheeppox virus or goatpox virus strains have also been tested and used to protect cattle against LSD. Sheeppox virus-based heterologous vaccines usually contain higher doses of virus than when administered to sheep. Although safe, their effectiveness in protecting cattle against LSD is inferior compared to homologous vaccines (Ben-Gera *et al*., 2015; Zhugunissov *et al*., 2020). Heterologous vaccines containing goatpox virus strains for use in cattle against LSD have been developed more recently. One such vaccine based on the Gorgan strain provided protection under experimental conditions comparable to homologous vaccines (Gari *et al*., 2015). On the other hand, a goat pox vaccine based on an attenuated Uttarkashi goatpox virus strain performed suboptimally under field conditions in India (Naveem *et al*., 2023), indicating that further research is warranted before asserting that all goatpox virus-based vaccines induce protection equal to homologous vaccines in cattle against LSD.

In addition, homologous inactivated vaccines against LSD have been developed and tested (Haegeman *et al*., 2023; Hamdi *et al*., 2020; Wolff *et al*., 2022). These vaccines are reported to be safe and efficacious. They however require a booster vaccination one month after primo-vaccination and then every 6 months thereafter, based on the fact that the duration of immunity is shorter than 1 year (Haegeman *et al*., 2023).

None of the commercial vaccines currently available has practical DIVA capacity. This problem may be resolved in the future by introducing new types of vaccines (e.g. vector-vaccines, subunit vaccines, mRNA vaccines) that are at various stages of development and evaluation.

2. Outline of production of LSD vaccines

The production of vaccines, including LSD vaccines, starts within research and development (R&D) facilities where vaccine candidates are produced and tested in preclinical studies to demonstrate the quality, safety and efficacy of the product.

Minimum requirements for different production stages of veterinary vaccines are available in different chapters of the *Terrestrial Manual.* These are intended to be used in combination with country-specific regulatory requirements for vaccine production and release. Here we outline the most important requirements for the production of live and inactivated LSD vaccines. Full requirements are available in Chapter 1.1.8 *Principles of veterinary vaccine production,* Chapter 2.3.3 *Minimum requirements for the organisation and management of a vaccine manufacturing facility* and Chapter 2.3.4 *Minimum requirements for the production and quality control of vaccine,* and other regulatory documentation.

2.1. Quality assurance

Facilities for manufacturing LSD vaccines should operate in line with the concepts of good laboratory practice (GLP) and good manufacturing practice (GMP) to produce high quality products. Quality risk management and quality control with adequate documentation management, as an integral part of the production process, have to be in place. In case some activities of the production process are outsourced, those should also be appropriately defined, recorded and controlled.

The vaccine production process (Outline of Production) should be documented in a series of standard operating procedures (SOPs), or other documents describing the manufacturing of each batch and the final product (including starting materials to be used, manufacturing steps, in-process controls and controls on the final product). Detailed requirements for documentation management in the process of vaccine production are available in Chapter 2.3.3.

A completed Outline of Production is to be enclosed in a vaccine candidate dossier and used for the evaluation of the production process and product by regulatory bodies.

2.2. Process validation

The dossier with the enclosed Outline of Production for the vaccine candidate has to be submitted for regulatory approval, so it can be assessed and authorised by the competent authority to ensure compliance with local regulatory requirements. Among others, data on quality, safety, and efficacy will be assessed. The procedures necessary to obtain these data are described in the subsequent sections.

National regulatory authorities might also require official control authority re-testing (check testing) of final products and batches in government laboratories or an independent batch quality control by a third party.

3. Requirements for LSD vaccine candidates and batch production

3.1. Requirements for starting materials

Live attenuated vaccines (LAV) and inactivated vaccines (IV) for LSD are produced using the system of limited and controlled passages of master seed and working seed virus and cell banks with a specified maximum. This approach aims to prevent possible and unwanted drift of properties of seed virus and cells that might arise from repeated passaging.

3.1.1. Characteristics of the seed virus

Master seed virus is a quantity of virus of uniform composition derived from an original isolate, passaged for a documented number of times and distributed into containers at one time and stored adequately to ensure stability (via freezing or lyophilisation). Selection of master seed viruses (MSVs) should ideally be based on their ease of growth in cell culture, virus yield, and in accordance with the regional epidemiological importance. Also, measures to minimise transmissible spongiform encephalopathies (TSE) contamination should be taken into account (see Section C.3.5.1 *Purity tests*).

For each seed strain selected for LSD vaccine production, the following information should be provided:

- Historical record: geographical origin, animal species from which the virus was recovered, isolation procedure, tissue culture or animal passage history

- Identity: species and strain identification using DNA sequencing
- Purity: the absence of bacteria, fungi, mycoplasma, and other viruses (see Chapter 1.1.9 *Tests for sterility and freedom from contamination of biological materials intended for veterinary use*)
- Safety (overdose, one/repeated dose tests, and reversion to virulence tests) (see Section C.3.3 *Vaccine safety*)
- Efficacy data, linked to a specified (protective) dose (see Section C.3.4 *Vaccine efficacy*)
- Stability

Each master seed strain selected for production of live attenuated LSD vaccines must remain attenuated after further passage in animals (see Section C.3.3. *Vaccine safety*), produce minimal clinical reaction when given via the recommended route, provide complete protection against challenge with virulent field strains, and is ideally not transmissible.

A quantity of master seed virus should be prepared and stored to be further used for the preparation of working seeds and production seeds. Working seed viruses may be expanded in one or more (but, limited) cell culture passages from the master seed stock and used to produce vaccine batches. This approach and limitation of seed virus passaging will assist in maintaining uniformity and consistency in production.

3.1.2. Master cell stocks

The production process of LSD vaccines ideally employs an established master cell stock (MCS) system with defined lowest and highest cell passage to be used to grow the vaccine virus. Primary cells derived from normal tissues can be used in the production process, but the use of primary cells has an inherently higher risk of introducing extraneous agents compared with the use of established (well characterised) cell lines and should be avoided where alternative methods of producing effective vaccines exist. For each MCS, manufacturers should demonstrate:

- MCS identity
- genetic stability by subculturing from the lowest to the highest passage used for production
- stable MCS karyotype with no evidence of subclonality
- freedom from oncogenicity or tumorigenicity by using *in-vivo* studies using the highest cell passage that may be used for production
- purity of MCSs from extraneous bacteria, fungi, mycoplasma, and viruses
- implemented measures to lower TSE contamination risk (see Section C.3.5.1 *Purity tests*).

3.2. Method of vaccine manufacturing

3.2.1. LSD vaccine batch production

As already mentioned in the first paragraphs of Section C, all steps undertaken in the production of vaccine batches should be described and documented in the Outline of Production. The production of LAV and IV against LSD starts with the inoculation of the required number of working vials of seed virus reconstituted in appropriate medium onto a suitable primary or continuous cell line grown in suspension or monolayer in the exponential growth phase. At the time highest viral loads are present, sonication or repeated freeze–thawing is used to release the intracellular virus from the cytoplasm. The lysate may then be clarified using centrifugation to remove cellular debris, with retention of the supernatant.

An aliquot of the virus suspension is titrated to check the virus titre. For LAV, the virus-containing suspension is diluted to attain the dose at which the vaccine candidate will be evaluated or to at least the determined protective dose for approved vaccines and is then mixed with a suitable protectant and transferred to individually labelled bottles or bags for storage at low temperatures such as –80°C, or for freeze–drying. A written record of all the procedures followed must be kept for all vaccine batches.

3.2.2. Antigen inactivation process for the generation of inactivated LSD vaccines

Unlike LAV, inactivated LSD vaccines contain inactivated antigens in combination with adjuvants to strengthen the induced immune response after administration. The vaccine evaluation process described below needs to show the amount of antigen necessary to elicit a protective immune response. Currently, published data indicate that an inactivated vaccine originating from an LSDV virus stock with titre 10^4 cell culture infectious dose$_{50}$ (CCID$_{50}$)/ml before inactivation can be sufficient to induce an efficient immune response to prevent clinical disease, viremia and virus shedding after challenge of young cattle (Wolf *et al.,* 2022)

To monitor the inactivation process and the level of antigen inactivation, samples are taken at regular intervals during inactivation and titrated. Inactivation conditions and the length of initial and repeated exposure should be documented in detail since one or more factors during the process could influence the outcomes. The inactivation kinetics should reach a predefined target e.g. one remaining infectious unit per million doses (1×10^{-6} infectious units/dose) as suggested by APHIS (2013). The confirmatory testing of inactivation is performed on each vaccine lot and represents an important part of the inactivation process monitoring. In addition to all the procedures mentioned above, the inactivation procedure and tests demonstrating that antigen inactivation is complete and consistent must additionally be documented in the Outline of Production.

3.3. Vaccine safety

During the vaccine development process, vaccine safety must be evaluated in the target animal (target animal batch safety test –TABST) to demonstrate the safety of the dose intended for registration. The animals used in the safety testing should be representative (species, age and category [calves, heifers, bulls, cows.]) for all the animals for which the vaccine is intended. Vaccinated and control groups are appropriately acclimatised, housed and managed in line with animal welfare standards. Animal suffering has to be eliminated or reduced and euthanasia is recommended in moribund animals.

Essential parameters to be evaluated in safety studies are local and systemic reactions to vaccination, including local reactions at the site of administration, fever, effect on milk production, and induction of a 'Neethling' response. The effect of the vaccine on reproduction needs to be evaluated where applicable.

A part of the safety evaluation of LAV and IV can be performed during the efficacy trials (see Section C.3.4 *Vaccine efficacy*) by measuring local and systemic responses following vaccination and before challenge.

Guidelines for safety evaluation are provided by the European Medicine Agency (EMEA) in VICH GL44: TABST for LAV and IV (EMEA, 2009). Safety aspects of LAV and IV against LSD to be evaluated are:

3.3.1. Overdose test for LAV

Local and systemic responses should be measured following an overdose test whereby 10× the maximum vaccine titre is administered. If the maximum vaccine titre is not specified, 10× the minimum vaccine titre can be applied in multiple injection sites. Ideally, the 10× dose is dissolved in the 1× dose volume of the adjuvants or diluent. Generally, eight animals per group should be used (EMEA, 2009).

3.3.2. One dose and repeat dose test

This aims to test the safety of the vaccine dose applied in the vaccination regime intended for registration. LAV LSD vaccines require one dose per year, while inactivated LSD vaccines require a booster dose in addition to the primary dose. The minimal recommended interval between administrations is 14 days.

Generally, eight animals per group should be used unless otherwise justified (EMEA, 2009). For each target species, the most sensitive breed, age and sex proposed on the label should be used. Seronegative animals should be used. In cases where seronegative animals are not reasonably available, alternatives should be justified.

3.3.3. Reversion to virulence tests

Live attenuated vaccines inherently carry the risk of vaccine virus reverting to virulence when repeated passages in a host species could occur due to shedding and transmission from vaccinated animals to contact animals. LAV LSD vaccines should therefore be tested for non-reversion to virulence by means of passage studies. Vaccine virus (MSV, not the finished vaccine) is inoculated in a group of target animals of susceptible age via the natural route of infection or the route that is most likely to result in infection. The vaccine virus is subsequently recovered from tissues or excretions and is used directly to inoculate a further group of animals. After not less than four passages (see chapter 1.1.8), i.e. use of a total of five groups of animals, the re-isolate must be fully characterised, using the same procedures used to characterise the master seed virus.

3.3.4. Environmental consideration

This includes the evaluation of the ability of LAV LSD vaccines to be shed, to spread and to infect contact target and non-target animals, and to persist in the environment.

3.4. Vaccine efficacy

Data enclosed in the vaccine candidate dossier should support the efficacy of the vaccine in each animal species for each vaccination regimen that is described in the product label recommendation. This includes studies regarding the onset of protection when claims for onset are made and for the duration of immunity. Efficacy studies should be conducted with the vaccine candidate that has been produced at the highest passage level permitted for vaccine production as specified in the Outline of Production.

Efficacy (and safety) should be demonstrated in vaccination–challenge studies using representative (by species, age and category) seronegative healthy animals for which the vaccine is intended and which are tested negative for standard viral pathogens.

An example of a vaccination-challenge test set-up is outlined here. The group numbers mentioned can be varied if statistically justified. Thirteen animals are placed in a high containment large animal unit and are divided into two groups:

- single/repeated dose test group (n=8) – animals inoculated with the vaccine dose and route intended for registration (in case of an IV against LSD, a booster dose should follow primary vaccination after minimum 14 days).
- control group (n=5) – non-vaccinated animals

Throughout the *in-vivo* study, all animals are clinically examined and rectal temperatures recorded. Blood, serum and swab samples are regularly collected and subjected to laboratory testing. On day 21 after the vaccination with a LAV or after the booster vaccination for an IV, the animals in both groups are challenged with a known virulent LSDV strain. The challenge virus solution should be of known titre and tested free from extraneous viruses. Experience obtained from previous animal experiments indicates that a dose of challenge virus between $10^{4.0}$ and $10^{6.5}$ $TCID_{50}$ produces clinical disease in about half of the susceptible experimental cattle (Tuppurainen *et al*., 2021).

The clinical response following challenge is recorded over a period of 14 days. No clinical signs should occur in the vaccinates, other than a local reaction at the site of inoculation. At least one animal in the unvaccinated control group should develop the typical clinical signs of LSD. Although a generalised disease with skin nodules may not be seen in all the unvaccinated control animals based on the knowledge that the outcome of a LSDV infection can range from inapparent to severe, at the very least a large local reaction is to be expected.

Clinical and laboratory results will enable assessment of the safety and efficacy of the LSD vaccine candidate and the induced immune responses. Serum samples collected at different time points during the trial can be examined to study seroconversion against selected viral diseases that could have contaminated the vaccine.

3.5. Batch/serial tests before release for distribution

Quality tests on MSV and safety and efficacy tests on vaccine candidates are performed during the evaluation process for new LSD vaccines. Once vaccines are approved to be used in the field, it remains important to verify the quality of each vaccine batch produced. An independent batch quality control assessment may be warranted or requested by national or international regulatory authorities.

3.5.1. Purity test

Purity is defined by the absence of different contaminants (bacteria, fungi, mycoplasma, and other viruses; see full details in chapter 1.1.9) in the vaccine and its associated diluent/adjuvants. Virus isolation and bacterial culture tests can be used to show freedom from live competent replicating microorganisms, but molecular methods are more rapid and sensitive, but positives can be caused by genome fragments and incompetent replicating microorganisms.

Besides the contaminants mentioned above, manufacturers should demonstrate implemented measures to minimise the risk of TSE contamination in ingredients of animal origin such as:

- all ingredients of animal origin in production facilities are from countries recognised as having the lowest possible risk of bovine spongiform encephalopathy
- tissues or other substances used are themselves recognised as being of low or nil risk of containing TSE agents

3.5.2. Identity tests

In addition to identity tests performed on the MSV, the identity tests on final batches aim to demonstrate the presence of only the selected capripoxvirus species and strain in the vaccine as indicated in the Outline of Production and the absence of other strains or members of the genus and any other viral contaminant that might arise during the production process. Identity testing could be assured by using appropriate tests (e.g. PCRs, sanger sequencing, next-generation sequencing).

3.5.3. Potency tests

Standard requirements for potency tests can be found in CFR Title 9 part 113, in the European Pharmacopoeia, and in this Terrestrial Manual.

3.5.3.1. Live vaccines

The potency of LAV against LSD can be measured by means of virus titration. The virus titre must, as a rule, be sufficiently greater than that shown to be protective in the efficacy test for the vaccine candidate. This will ensure that at any time prior to the expiry date, the titre will be at least equal to the evaluated protective titre. The titres of currently available commercial homologous LSD vaccines range between 10^3 and 10^4 infectious units/dose (Tuppurainen *et al*., 2021).

3.5.3.2. Inactivated LSD vaccines

For inactivated LSD vaccines, potency tests are performed using vaccination–challenge efficacy studies in animal hosts (see Section C.3.4. *Vaccine efficacy*).

3.5.4. Safety/efficacy

Safety and efficacy testing is undertaken during the evaluation process of the vaccine candidate, and also needs to be performed on a number of vaccine batches until robust data are generated in line with international and national regulations. Afterwards, when using a seed lot system in combination with strict implementation of GMP standards and depending on local regulations, TABST could be waived as described in VICH50 and VICH55, providing the titer has been ascertained using potency testing. Batches or serials are considered satisfactory if local and systemic reactions to vaccination are in line with those described in the dossier of the vaccine candidate and product literature.

3.5.4.1. Field safety/efficacy tests

Field testing of two or more batches should be performed on all animal categories for which the product is indicated before release of the product for general use (see chapter 1.1.8). The aim of these studies is to demonstrate the safety and efficacy of the product under normal field conditions of animal care and use in different geographical locations where different factors may influence product performance. A protocol for safety/efficacy testing in the field has to be developed with defined observation and recording procedures. However, it is generally more difficult to obtain statistically significant data to demonstrate efficacy under field conditions. Even when properly designed, field efficacy studies may be inconclusive due to uncontrollable outside influences.

3.5.4.2. Duration of Immunity

The duration of immunity (DOI) following vaccination should be demonstrated via challenge or the use of a validated serology test. Efficacy testing at the end of the claimed period of protection should be conducted in each species for which the vaccine is indicated or the manufacturer should indicate that the DOI for that species is not known. Likewise, the manufacturer should demonstrate the effectiveness of the recommended booster regime in line with these guidelines, usually by measuring the magnitude and kinetics of the serological response observed.

4. Post-market studies

4.1. Stability

Stability testing shall be carried out as specified in Annex II of Regulation (EU) 2019/6 and in the Ph. Eur. 0062: Vaccines for veterinary use, on not fewer than three representative batches providing this mimics the full-scale production described in the application. At the end of shelf-life, sterility has to be re-evaluated using sterility testing or by showing container closure integrity. Multiple batches of the vaccine should be re-titrated periodically throughout the shelf-life period to determine the vaccine stability.

4.2. Post-marketing surveillance

After release of a vaccine, its performance under field conditions should continue to be monitored by competent authorities and by the manufacturer itself. Not all listed adverse effects may show up in the clinical trials performed to assess safety and efficacy of the vaccine candidate due to the limited number of animals used. Post-marketing surveillance studies can also provide information on vaccine efficacy when used in normal practice and husbandry conditions. For example, such studies may shed light on the duration of induced immunity, and information surrounding ecotoxicity.

First, a reliable reporting system should be in place to collect consumer complaints and notifications of adverse reactions. Secondly, post-marketing surveillance should be established to investigate whether the reported observations are related to the use of the product and to identify, at the earliest stage, any serious problem that may be encountered from its use and that may affect its future uptake. Vaccinovigilance should be an on-going and integral part of all regulatory programmes for LSD vaccines, especially for live vaccines.

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NB: There are WOAH Reference Laboratories for lumpy skin disease (please consult the WOAH Web site: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact WOAH Reference Laboratories for any further information on diagnostic tests, reagents and vaccines for lumpy skin disease

NB: FIRST ADOPTED IN 1989. MOST RECENT UPDATES ADOPTED IN 2024.