

## CHAPTER 3.8.11.

# SHEEP POX AND GOAT POX

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### SUMMARY

Sheep pox and goat pox are contagious, viral diseases of sheep and goats characterised by fever, generalised papules or nodules, vesicles (rarely), internal lesions (particularly in the lungs), and death. Both diseases are caused by strains of capripoxvirus, all of which can infect sheep and goats. Although most of the strains examined cause more severe clinical disease in either sheep or goats, some strains have been isolated that are equally pathogenic in both species.

Sheeppox virus (SPPV) and goatpox virus (GTPV) are the causative agents of sheep pox and goat pox, and with lumpy skin disease virus (LSDV) make up the genus *Capripoxvirus* in the family *Poxviridae*. Sheep pox and goat pox are endemic in Africa north of the Equator, the Middle East and Asia, while some parts of Europe have experienced outbreaks recently. See WAHIS (<https://wahis.woah.org/#/home>) for recent information on distribution at the country level.

**Identification of the agent:** Laboratory confirmation of capripoxvirus is most rapid using the polymerase chain reaction (PCR) method in combination with a clinical history consistent with generalised capripoxvirus infection. Isolation of the virus is possible as capripoxviruses will grow on tissue culture of ovine, caprine or bovine origin, although field isolates may require up to 14 days to grow or require one or more additional tissue culture passage(s). The virus causes intracytoplasmic inclusions that can be clearly seen using haematoxylin and eosin staining. The antigen can also be detected in tissue culture using specific sera and immunoperoxidase or immunofluorescence techniques. Capripoxvirus antigen and inclusion bodies may be seen in stained cryostat or paraffin sections of biopsy or post-mortem lesion material.

**Serological tests:** The virus neutralisation test is the most specific serological test. The indirect immunofluorescence test is less specific due to cross-reactions with antibody to other poxviruses. Western blotting using the reaction between the P32 antigen of capripoxvirus with test sera is both sensitive and specific, but is expensive and difficult to carry out. An enzyme-linked immunosorbent assay (ELISA) has been developed and validated to detect antibodies to capripoxviruses, however it cannot differentiate between SPPV, GTPV and LSDV.

**Requirements for vaccines:** Live and inactivated vaccines have been used for the control of capripoxviruses. All strains of capripoxvirus so far examined share a major neutralisation site and some will cross protect. Inactivated vaccines give, at best, only short-term immunity.

### A. INTRODUCTION

The *Capripoxvirus* genus, in the family *Poxviridae*, consists of three species – lumpy skin disease virus (LSDV), which causes disease in cattle only (see Chapter 3.4.12), and sheeppox virus (SPPV) and goatpox virus (GTPV), which cause sheep pox and goat pox, respectively. Sheep pox and goat pox are characterised by disseminated cutaneous nodules and up to 100% mortality in fully naïve of sheep and goats. In indigenous animals, generalised disease and mortality are less common, although they are seen where disease has been absent from an area or village for a period of time, when intensive husbandry methods are introduced, or in association with other disease agents, such as peste des petits ruminants virus or foot and mouth disease virus. Sheep pox and goat pox are major constraints to the introduction of exotic breeds of sheep and goats to endemic areas, and to the development of intensive livestock production.

Strains of SPPV and GTPV can pass between sheep and goats, although most cause more severe clinical disease in their homologous host species. SPPV and GTPV are transboundary diseases that regularly spread into adjacent, non-endemic areas. Sheep pox and goat pox are endemic in Africa north of the Equator and parts of the Middle East and Asia (see WAHIS for most up-to-date information on distribution: <https://wahis.woah.org/#/home>). Outbreaks have been reported in non-endemic countries of Asia, Europe and the Middle East.

The incubation period of sheep pox and goat pox is between 8 and 13 days following contact between infected and susceptible animals. It may be as short as 4 days following experimental infection by intradermal inoculation. Some breeds of European sheep, such as Soay, may die of acute infection before the development of skin lesions. In other breeds there is an initial rise in rectal temperature to above 40°C, followed in 2–5 days by the development of, at first, macules – small circumscribed areas of hyperaemia, which are most obvious on unpigmented skin – and then of papules – hard swellings of between 0.5 and 1 cm in diameter – which may cover the body or be restricted to the groin, axilla and perineum. Papules may be covered by fluid-filled vesicles, but this is rare. Some researchers have distinguished between a vesicular and nodular form of sheep pox and goat pox (Zro *et al.*, 2014b).

Within 24 hours of the appearance of generalised papules, affected animals develop rhinitis, conjunctivitis and enlargement of all the superficial lymph nodes, in particular the prescapular lymph nodes. Papules on the eyelids cause blepharitis of varying severity. As the papules on the mucous membranes of the eyes and nose ulcerate, so the discharge becomes mucopurulent, and the mucosae of the mouth, anus, and prepuce or vagina become necrotic. Breathing may become laboured and noisy due to pressure on the upper respiratory tract from the swollen retropharyngeal lymph nodes, due to the developing lung lesions.

If the affected animal does not die in this acute phase of the disease, the papules start to become necrotic from ischaemic necrosis following thrombi formation in the blood vessels at the base of the papule. In the following 5–10 days the papules form scabs, which persist for up to 6 weeks, leaving small scars. The skin lesions are susceptible to fly strike, and secondary pneumonia is common. Anorexia is not usual unless the mouth lesions physically interfere with feeding. Abortion is rare.

On post-mortem examination of the acutely infected animal, the skin lesions are often less obvious than on the live animal. The mucous membranes appear necrotic and all the body lymph nodes are enlarged and oedematous. Papules, which may be ulcerated, can usually be found on the abomasal mucosa, and sometimes on the wall of the rumen and large intestine, on the tongue, hard and soft palate, trachea and oesophagus. Pale areas of approximately 2 cm in diameter may occasionally be seen on the surface of the kidney and liver, and have been reported to be present in the testicles. Numerous hard lesions of up to 5 cm in diameter are commonly observed throughout the lungs, but particularly in the diaphragmatic lobes.

The clinical signs and post-mortem lesions vary considerably with breed of host and strain of capripoxvirus. Indigenous breeds are less susceptible and frequently show only a few lesions, which could be confused with insect bites or contagious pustular dermatitis. However, lambs that have lost their maternally derived immunity, animals that have been kept isolated and animals brought into endemic areas from isolated villages, particularly if they have been subjected to the stress of moving long distances and mixing with other sheep and goats, and their pathogens, can often be seen with generalised and sometimes fatal capripoxvirus infections. Invariably there is high mortality in unprotected imported breeds of sheep and goats following capripoxvirus infection. Surviving animals clear the infection, as there is no evidence of persistently infected animals. Capripoxvirus is not infectious to humans. Capripoxvirus is inactivated at 56°C for 2 hours or 65°C for 30 minutes. The virus survives between pH 6.6–8.6. It is susceptible to highly alkaline or acid pH. The virus is sensitive to various chemicals: sodium dodecyl sulphate, ether 20%, chloroform, formalin 1%, sodium 2%, iodine compounds, Virkon 2%, quaternary ammonium (0.5%), and phenol 2% for 15 minutes.

## B. DIAGNOSTIC TECHNIQUES

*Table 1. Test methods available for diagnosis of sheep pox and goat pox 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 |
| <b>Identification of the agent</b>  |                                   |  |                                    |                                |  |   |
| Virus isolation                     | +                                 | ++   | +                                  | +++                            | +                                      | –   |
| IFAT                                | +                                 | +  | +                                  | ++                             | +                                      | –   |
| IHC                                 | +                                 | +  | +                                  | ++                             | +                                      | –   |
| PCR                                 | ++                                | +++  | ++                                 | +++                            | ++                                     | –   |
| <b>Detection of immune response</b> |                                   |  |                                    |                                |  |   |
| VNT                                 | ++                                | ++   | ++                                 | ++                             | ++                                     | ++  |
| IFAT                                | +                                 | +  | +                                  | +                              | +                                      | +   |
| ELISA                               | ++                                | ++   | ++                                 | ++                             | ++                                     | ++  |

Key: +++ = recommended for this purpose; ++ recommended but has limitations;

+ = suitable in very limited circumstances; – = not appropriate for this purpose.

IFAT = indirect fluorescent antibody test; IHC = immunohistochemistry; PCR = polymerase chain reaction;

VNT = virus neutralisation; ELISA = enzyme-linked immunosorbent assay.

### 1. Identification of the agent

#### 1.1. Specimen collection and submission

Material for virus isolation should be collected by biopsy or at post-mortem from skin papules, lung lesions or lymph nodes. Samples for virus isolation should be collected within the first week of the occurrence of clinical signs, before the development of neutralising antibodies. Samples for genome detection by polymerase chain reaction (PCR) may be collected before or after the development of neutralising antibody responses. In addition to epithelial lesions, nasal and buccal swabs can be collected because the virus will be present in nasal and saliva discharges. Buffy coat from blood collected into EDTA (ethylene diamine tetra-acetic acid) during the viraemic stage of capripoxvirus infection (before generalisation of lesions or within 4 days of generalisation), can also be used for virus isolation.

Samples for histology should include tissue from the surrounding area and should be placed immediately following collection into ten times the sample volume of 10% formalin or neutral buffered 10% formal saline.

Blood samples, for virus isolation from the buffy coat, should be collected in tubes containing anticoagulant, placed immediately on ice and processed as soon as possible. In practice, the blood 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 and dry scabs for virus isolation, and genome detection should preferably 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 that the transport medium does not penetrate the central part of the biopsy, which should be used for virus isolation/detection.

## 1.2. Virus isolation

Lesion material for virus isolation and genome detection is homogenised. The following is an example of one technique for homogenisation: The tissue is minced using sterile scissors and forceps, and then macerated in a steel ball bearing mixer mill or ground with a sterile pestle in a mortar with sterile sand and an equal volume of sterile phosphate buffered saline (PBS) or serum-free Modified Eagle's Medium (MEM) containing sodium penicillin (1000 international units [IU]/ml), streptomycin sulphate (1 mg/ml), mycostatin (100 IU/ml) or fungizone (2.5 µg/ml) and neomycin (200 IU/ml). The homogenised suspension is freeze–thawed three times and then partially clarified by centrifugation 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, however, the amount of virus in the supernatant might be reduced. Buffy coats may be prepared from 5–8 ml unclotted blood by centrifugation at 600 *g* for 15 minutes; the buffy coat is 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 of 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 using a density gradient.

Capripoxvirus will grow in tissue culture of bovine, ovine or caprine origin, although primary or secondary cultures of lamb testis (LT) or lamb kidney (LK) cells are considered to be the most susceptible. Care needs to be taken to ensure they are not contaminated with viruses such as bovine viral diarrhoea virus (see Chapter 1.1.9 *Tests for sterility and freedom from contamination of biological materials intended for veterinary use*). Madin–Darby bovine kidney (MDBK) cells have been shown to be suitable for capripoxvirus isolation (Fay *et al.*, 2020). The following is an example of an isolation technique: either 1 ml of buffy coat cell suspension or 1 ml of clarified biopsy preparation supernatant is inoculated on to a 25 cm<sup>2</sup> tissue culture flask of appropriate cells at 90% confluence, and the supernatant is allowed to adsorb for 1 hour at 37°C. 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, flying cover-slips, or tissue culture microscope slides, can also be infected.

The flasks should be examined daily for 7–14 days for evidence of cytopathic effect (CPE). Contaminated flasks should be discarded. 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 4 days after infection; over the following 4–6 days these expand to involve the whole cell sheet. If no CPE is apparent by day 7, the culture should be freeze–thawed three times, and clarified supernatant inoculated on to fresh cell cultures. 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 indicative of poxvirus infection. Syncytia formation is not a feature of capripoxvirus infection. If the CPE is due to capripoxvirus infection of the cell culture, it can be prevented or delayed by inclusion of specific anti-capripoxvirus serum in the medium; this provides a presumptive identification of the agent. Some strains of capripoxvirus have been adapted to grow on African green monkey kidney (Vero) cells, but these cells are not recommended for primary isolation.

## 1.3. 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 is given below:

Material from the original tissue suspension is prepared for transmission electron microscope examination, prior to centrifugation, by floating a 400-mesh hexagon electron microscope grid, with piliform-carbon substrate activated by glow discharge in pentylamine vapour, on to 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 capripoxvirus 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, no orthopoxvirus causes lesions in sheep and goats. However, capripoxvirus is distinguishable from the virions of parapoxvirus, that cause contagious pustular dermatitis, as they are smaller, oval in shape, and each is covered in a single continuous tubular element, which appears as striations over the virion.

#### **1.4. Histopathology**

Material for histopathology and immunohistochemistry should be prepared by standard techniques (Parvin *et al.*, 2022). Following preparation and staining with haematoxylin and eosin (H&E), a number of sections should be examined by light microscopy. On histological examination, the most striking aspects of acute-stage skin lesions are a massive cellular infiltrate, vasculitis and oedema. Early lesions are characterised by marked perivascular cuffing. Initially infiltration is by macrophages, neutrophils and occasionally eosinophils, and as the lesion progresses, by more macrophages, lymphocytes and plasma cells. A characteristic feature of all capripoxvirus infections is the presence of variable numbers of 'sheep pox cells' in the dermis. These sheep pox cells can also occur in other organs where microscopic lesions of sheep and goat pox are present. These cells are large, stellate cells with eosinophilic, poorly defined intracytoplasmic inclusions and vacuolated nuclei. Vasculitis is accompanied by thrombosis and infarction, causing oedema and necrosis. Epidermal changes consist of acanthosis, parakeratosis and hyperkeratosis. Changes in other organs are similar, with a predominant cellular infiltration and vasculitis. Lesions in the upper respiratory tract are characterised by ulceration.

Immunohistochemistry will show capripox virus antigen infiltrated macrophages throughout the subcutis. The capripox virus antigen can occasionally be detected in hair follicle epithelial cells, the endothelium and smooth muscle cells of the blood vessels, and histiocytic cells (Parvin *et al.*, 2022).

#### **1.5. Immunological methods**

##### **1.5.1. Fluorescent antibody tests**

Capripoxvirus antigen can also 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 sheep or goat sera is subject to high background colour and nonspecific reactions. However, a direct conjugate can be prepared from sera from convalescent sheep or goats or from rabbits hyperimmunised with purified *Capripoxvirus*. Uninfected tissue culture should be included as a negative control because cross-reactions, due to antibodies to cell culture antigens, can cause problems. The fluorescent antibody tissue section technique has also been used on cryostat-prepared slides.

#### **1.6. Nucleic acid recognition methods**

Amplification methods for detection of *Capripoxvirus* DNA are both specific and sensitive throughout the course of disease, including before and after the emergence of antibody responses. These methods include conventional PCR, real-time PCR, and most recently loop-mediated isothermal amplification (LAMP). Nucleic acid recognition methods can be used to detect fragments of the *Capripoxvirus* genome in biopsy, swab, blood, semen or tissue culture samples. It is important that nucleic acid extraction and PCR amplification methods are validated for the sample matrix being tested.

##### **1.6.1. Conventional PCR methods**

Several conventional PCR methods have been reported with varying specificity for capripoxviruses in general, SPPV, or GTPV (Heine *et al.*, 1999; Ireland & Binopal, 1998; Zro *et al.*, 2014a). A conventional PCR assay that differentiates GTPV and LSDV from SPPV has been described (Lamien *et al.*, 2011a). Conventional PCR methods are particularly useful for obtaining sufficient genetic material necessary for species identification by subsequent sequence and phylogenetic analysis (Le Goff *et al.*, 2009).

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).

### 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 & Binopal, 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<sub>2</sub> (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.

### 1.6.2. Real-time PCR methods

Several highly sensitive and specific fluorescent detection-based real-time PCR methods have been developed and validated (Balinsky *et al.*, 2008; Bowden *et al.*, 2008; Das *et al.*, 2012; Stubbs *et al.*, 2012). Each test detects a small conserved genetic locus within the capripoxvirus genome, but these methods do not discriminate between SPPV, GTPV or LSDV. Real-time PCR methods for direct capripoxvirus species differentiation without the need for gene sequencing have been described (Haegeman *et al.*, 2013; Gelaye *et al.*, 2013; Lamien *et al.*, 2011b; Wolff *et al.*, 2021).

The real-time PCR method described below is a rapid, sensitive and specific method for the detection of the genomic DNA from SPPV, GTPV or LSDV. This assay is not designed to differentiate the capripoxvirus species.

#### DNA extraction from blood, tissue and semen

A number of DNA extraction kits are commercially available for the extraction of template DNA for real-time PCR. Manufacturer's instructions should always be followed while using commercial extraction kits. WOAH Reference Laboratories can be contacted for advice on suitable commercial kits.

#### Real-time PCR

- i) The real-time PCR method outlined below uses the primers and probe described by Bowden *et al.* (2008), and further validated by Stubbs *et al.* (2012). Cycling conditions and reagent concentrations can be altered to ensure optimal performance in individual laboratories.
- ii) Forward and reverse primers should be prepared at concentrations of 20  $\mu$ M. A minor groove binder (MGB) hydrolysis probe should be prepared at a concentration of 10  $\mu$ M.

Forward primer: 5'-AAA-ACG-GTA-TAT-GGA-ATA-GAG-TTG-GAA-3'

Reverse primer: 5'-AAA-TGA-AAC-CAA-TGG-ATG-GGA-TA-3'

Probe: 5'-FAM-TGG-CTC-ATA-GAT-TTC-CT-MGB-3'

- iii) Mastermix is prepared by combining 10  $\mu$ l of 2  $\times$  real-time PCR mastermix with 0.4  $\mu$ l of forward primer, 0.4  $\mu$ l of reverse primer, 0.5  $\mu$ l of probe and 6.7  $\mu$ l of RNase free water per reaction.
- iv) Add 2  $\mu$ l of extracted DNA to 18  $\mu$ l of mastermix in a 96-well PCR plate or PCR strip and perform real-time PCR according to the example given below or similar method:
- v) 95°C for 10 minutes followed by 45 cycles of 95°C for 15 seconds, 60°C for 60 seconds. Fluorescence detection should be performed at the end of each cycle.
- vi) Following completion of the real-time PCR, a cycle threshold ( $C_T$ ) should be set. Samples with  $C_T$  values less than 35 are considered positive. Samples with a  $C_T$  value greater than 35 but less than 45 are considered inconclusive and require further investigation. Samples that do not yield a  $C_T$  value, i.e. the amplification curve does not cross the threshold, are considered negative.

#### 1.6.3. Isothermal genome amplification

Molecular tests using LAMP to detect capripoxvirus genomes are reported to provide sensitivity and specificity similar to real-time PCR with a simpler method and at lower cost (Das *et al.*, 2012; Murray *et al.*, 2013). Field validation of the Das *et al.* (2012) LAMP assay has been further reported (Omoga *et al.*, 2016) and a combination of this universal capripoxvirus test with two additional LAMP assays was reported to differentiate GTPV from SPPV (Zhao *et al.*, 2014).

## 2. Serological tests

Detectable levels of antibodies develop 1 week after the animal shows clinical signs. The highest antibody levels are detected within 1–2 months after infection is detected.

### 2.1. Virus neutralisation

A test serum can either be titrated against a constant titre of capripoxvirus (100 TCID<sub>50</sub> [50% tissue culture infective dose]) or a standard capripoxvirus 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 use of 100 TCID<sub>50</sub>, the neutralisation index is the preferred method, 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) 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 without serum is placed in columns 11 and 12 and to all wells of 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<sub>50</sub> 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<sub>50</sub> per ml (equivalent to  $\log_{10}$  3.7; 2.7; 2.2; 1.7; 1.2; 0.7; 0.2 TCID<sub>50</sub> 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 toxicity 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 starting at 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, the final reading is taken on day 9, and the titre of virus in each duplicate titration is calculated according to 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  $\geq 1.5$  is positive. The test can be made more sensitive if serum from the same animal is examined before and after infection. Because immunity to capripoxvirus is predominantly cell mediated, a negative result, particularly following vaccination in which the response is necessarily mild, does not imply that the animal from which the serum was taken is not protected.

### 2.2. Indirect fluorescent antibody test

Capripoxvirus-infected tissue culture grown on flying 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 1/5, and positives are identified using an anti-sheep gamma-globulin conjugated with fluorescein isothiocyanate (Davies & Otema, 1978). Cross-reactions can occur with orf, bovine papular stomatitis virus and perhaps other poxviruses.

### 2.3. 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 (Chand *et al.*, 1994).

## 2.4. Enzyme-linked immunosorbent assay

Both in-house and commercial enzyme-linked immunosorbent assay (ELISAs) are available, but these tests cannot discriminate between antibodies to different capripoxviruses (LSDV or SPPV/GTPV).

## C. REQUIREMENTS FOR VACCINES [UNDER STUDY]

### 1. Background

#### 1.1. Rationale and intended use of the product

A variety of attenuated live and inactivated capripoxvirus vaccines has been used to provide protection against sheep pox and goat pox. All strains of capripoxvirus of ovine, caprine or bovine origin examined so far share a major neutralising site, so that animals recovered from infection with one strain are resistant to infection with any other strain (Capstick, 1961). Consequently, it is possible to use a single strain of capripoxvirus to protect both sheep and goats against all field strains of virus, regardless of whether their origin was in Asia or Africa (Kitching *et al.*, 1986; Kitching & Taylor, 1985). However, field evidence suggests some strains are quite host-specific and are used only in sheep against SPPV and only in goat against GTPV.

A number of strains of capripoxvirus have had widespread use as live vaccines (Davies & Mbugwa, 1985), for example the Romanian and RM-65 strains used mainly in sheep and the Mysore and Gorgan strains used in goats. The real identity of the commonly used Kenyan sheep and goat pox vaccine virus (KSGP) 0240 was recently shown to be actually LSDV (Tuppurainen *et al.*, 2014). Virus strain identity and attenuation properties must be ascertained and taken into consideration when selecting vaccine strains for use in cattle, sheep and goats. The protective dose depends on the vaccine strain used. Immunity in sheep and goats against capripoxvirus following vaccination with the 0240 strain lasts over a year and the Romanian strain gave protection for at least 30 months.

Killed vaccines produced from tissue culture contain only the intracellular mature virion form of the virus, and lack the less robust but biologically crucial extracellular enveloped virion form. As a result, the vaccine does not stimulate immunity against the extracellular enveloped virion, resulting in poor protection. Killed capripoxvirus vaccines provide, at best, only temporary protection.

### 2. Outline of production and minimum requirements for conventional vaccines

General requirements set for the facilities used for the production of vaccines and for the documentation and record keeping throughout the whole manufacturing process are described in Chapter 1.1.8 *Principles of veterinary vaccine production*. The documentation should include the standard operating procedures (SOP) for the method of manufacture and each step for the testing of cells and reagents used in the process, each batches and the final product.

#### 2.1. Characteristics of the seed

##### 2.1.1. Biological characteristics

A strain of capripoxvirus used for vaccine production must be accompanied by a history describing its origin and tissue culture or animal passage. It must be safe to use in all breeds of sheep and goats for which it is intended, including pregnant and young animals. It must be non-transmissible, remain attenuated after further tissue culture passage, and provide complete protection against challenge with virulent field strains for a minimum of 1 year. A quantity of master seed vaccine virus should be prepared and stored in order to provide a consistent working seed for regular vaccine production.

##### 2.1.2. Quality criteria (sterility, purity, freedom from extraneous agents)

Each master seed must be tested to ensure its identity and shown to be free from adventitious viruses, in particular pestiviruses, such as border disease and bovine viral diarrhoea virus, and free from contamination with bacteria, fungi and/or mycoplasmas. The general procedures for

sterility or purity tests are described in chapter 1.1.9. The master seed must also be safe and produce no clinical reaction in all breeds of sheep or goats when given by the recommended route and stimulate complete immunity to capripoxvirus in all breeds of sheep and goats for at least 1 year. The necessary safety and potency tests are described in Section C.2.2.4 *Final product batch tests*.

## 2.2. Method of manufacture

The method of manufacture should be documented as the Outline of Production.

### 2.2.1. Procedure

Vaccine seed should be lyophilised and stored in 2 ml vials at  $-20^{\circ}\text{C}$ . It may be stored wet at  $-20^{\circ}\text{C}$ , but when wet, is more stable at  $-70^{\circ}\text{C}$  or lower. The virus should be cultured in primary or secondary LT or LK cells of wool sheep origin for maximum yield. Vero cells may also be used with suitably adapted strains.

Vaccine batches are produced on fresh monolayers of secondary LT or primary LK cells. A vial of seed virus is reconstituted with GMEM or another appropriate medium and inoculated on to an LT or LK monolayer that has been previously washed with warm PBS, and allowed to adsorb for 15 minutes at  $37^{\circ}\text{C}$  before being overlaid with additional GMEM. After 4–6 days, there will be extensive (80–90%) CPE. The culture should be examined for any evidence of nonspecific CPE, medium cloudiness or change in medium pH. The culture is freeze–thawed three times, the suspension removed and centrifuged at 600 *g* for 20 minutes. A second passage may be required to produce sufficient virus for a production batch. Live vaccine may be produced on roller bottles.

The procedure is repeated and the harvests from individually numbered flasks are each mixed separately with an equal volume of sterile and chilled 5% lactalbumin hydrolysate and 10% sucrose, and transferred to individually numbered bottles for storage at  $-20^{\circ}\text{C}$ . Prior to storage, 0.2 ml is removed from each bottle for sterility control. An additional 0.2 ml is removed for virus titration; 2 ml pools composed of 0.2 ml samples taken from ten bottles are used. A written record of all the procedures must be kept for all vaccine batches.

Inactivated vaccines are produced, usually from unattenuated field strains of capripoxvirus, grown in tissue culture as described above, inactivated with 0.03% formaldehyde, and mixed with an equal volume of alhydrogel as adjuvant. Formaldehyde is no longer considered to be a suitable inactivant for certain viral vaccines because its mode of action cannot be guaranteed to be totally effective in inactivating all the live virus. This has not been fully investigated for capripoxvirus.

### 2.2.2. Requirements for substrate and media

The specification and source of all ingredients used in the manufacturing procedure should be documented and the freedom from extraneous agents: bacteria, fungi, mycoplasma and any other viruses should be tested. The detailed testing procedure is described in the chapter 1.1.9. The use of antibiotics must meet the requirements of the licensing authority.

### 2.2.3. In-process controls

#### i) Cells

Cells should be obtained from the testis or kidney of a healthy young lamb from a scrapie-free flock of a wool sheep breed. During cultivation, cells must be observed for any evidence of CPE, and for normal morphology (predominantly fibroblastic). They can usually be passaged successfully up to ten times. When used for vaccine production, uninfected control cultures should be grown in parallel and maintained for at least three additional passages for further observation. They should be checked for the presence of noncytopathic strains of bovine virus diarrhoea or border disease viruses by immunofluorescence or immunoperoxidase techniques. If possible, cells should be prepared and screened prior to vaccine production and stocked in 1–2 ml aliquots containing  $2 \times 10^7$  cells/ml in sterile 10% DMSO (dimethyl sulphoxide) and 90% FBS (fetal bovine serum) solution stored in liquid nitrogen.

ii) Serum

Bovine serum used in the growth or maintenance medium must be free from transmissible spongiform encephalopathies (TSEs) and antibody to capripoxvirus, and tested for contamination with pestivirus or any other viruses, extraneous bacteria, mycoplasma or fungi.

iii) Medium

Medium must be tested free from contamination with pestivirus or any other viruses, extraneous bacteria, mycoplasma or fungi.

iv) Virus

Seed virus and final vaccine must be titrated in tissue culture tubes or microtitre plates. Vaccine samples must be examined for the presence of adventitious viruses including cytopathic and noncytopathic strains of pestivirus, and should be mixed with a high-titre capripoxvirus-immune serum that has tested negative for antibody to pestivirus to prevent the vaccine virus itself interfering with the test. The vaccine bulk can be held at  $-20^{\circ}\text{C}$  or below until all sterility tests and titrations have been completed, at which time it should be freeze-dried in 1 ml aliquots in vials sufficient for 100 doses. The vaccine harvest diluted with lactalbumin hydrolysate and sucrose should have a minimum titre  $\log_{10}$  4.5 TCID<sub>50</sub> per ml after freeze-drying, equivalent to a field dose of  $\log_{10}$  2.5 TCID<sub>50</sub>. A further titration is carried out on five randomly chosen vials of the freeze-dried preparation to confirm the titre.

#### 2.2.4. Final product batch tests

i) Sterility/purity

Tests for sterility and freedom from contamination of biological materials intended for veterinary use may be found in chapter 1.1.9.

ii) Safety

The safety studies should be demonstrated by statistically valid vaccination studies using seronegative young sheep and goats of known susceptibility to capripox virus. The procedure described is suitable for vaccine strains such as O240 that are equally immunogenic in both sheep and goats. The choice of target animal should be adapted for strains with a more restricted host preference.

iii) Potency

Potency tests must be undertaken if the minimum immunising dose of the virus strain is not known. This is usually carried out by comparing the titre of a virulent challenge virus on the flanks of vaccinated and control animals. Following vaccination, the flanks of at least three animals and three controls are shaved of wool or hair.  $\log_{10}$  dilutions of the challenge virus are prepared in sterile PBS and six dilutions are inoculated intradermally (0.1 ml per inoculum) along the length of the flank; four replicates of each dilution are inoculated down the flank. An oedematous swelling will develop at possibly all 24 inoculation sites on the control animals, although preferably there will be little or no reaction at the four sites of the most dilute inocula. The vaccinated animals should develop an initial hypersensitivity reaction at sites of inoculation within 24 hours, which should quickly subside. Small areas of necrosis may develop at the inoculation site of the most concentrated challenge virus. The macule/papule is measured at between 8 and 10 days post-challenge. The titre of the challenge virus is calculated for the vaccinated and control animals; a difference of  $\log_{10}$  titre  $> 2.5$  is taken as evidence of protection.

## 2.3. Requirements for authorisation

### 2.3.1. Safety requirements

i) Target and non-target animal safety

The vaccine must be safe to use in all breeds of sheep and goats for which it is intended, including young and pregnant animals. It must also be non-transmissible, remain attenuated after further tissue culture passage.

Safety tests should be carried out on the final product of each batch as described in Section C.2.2.4.

The safety of the vaccine in non-target animals must have been demonstrated using mice and guinea-pigs as described in Section C.2.2.4. There should be no evidence of pathology caused by the vaccine.

ii) Reversion-to-virulence for attenuated/live vaccines

The selected final vaccine should not revert to virulence during a further passages in target animals.

iii) Environmental consideration

Attenuated vaccine should not be able to perpetuate autonomously in cattle, sheep or goat populations. Vaccines using the O240 strain should not be used in *Bos taurus* breeds. Strains of capripoxvirus are not a hazard to human health. There are no precautions other than those described above for sterility and freedom from adventitious agents.

### 2.3.2. Efficacy requirements

i) For animal production

The efficacy of the vaccine must be demonstrated in vaccination challenge experiment under laboratory conditions. As described in Section C.2.2.4.

Once the potency of the particular strain being used for vaccine production has been determined in terms of minimum dose required to provide immunity, it is not necessary to repeat this on the final product of each batch, provided the titre of virus present has been ascertained.

ii) For control and eradication

Vaccination is the only effective way to control the sheep pox and goat pox outbreaks in endemic countries. Unfortunately, currently no marker vaccines allowing the differentiation of infected from vaccinated animals are available.

Immunity to virulent field virus following vaccination of sheep or goats with the O240 strain lasts over 1 year, and protection against generalised infection following intradermal challenge lasts at least 3 years and is effective lifelong. The duration of immunity produced by other vaccine strains should be ascertained in both sheep and goats by undertaking controlled trials in an environment in which there is no possibility of field strains of capripoxvirus confusing the results. The inactivated vaccines provide immunity for less than 1 year, and for the reasons given at the beginning of this section, may not give immunity to the form of capripoxvirus usually associated with natural transmission.

### 2.3.3. Stability

All vaccines are initially given a shelf-life of 24 months before expiry. Real-time stability studies are then conducted to confirm the appropriateness of the expiry date. Multiple batches of the vaccine should be re-titrated periodically throughout the shelf-life to determine the vaccine variability.

Properly freeze-dried preparations of capripox vaccine, particularly those that include a protectant, such as sucrose and lactalbumin hydrolysate, are stable for over 25 years when stored at  $-20^{\circ}\text{C}$  and for 2–4 years when stored at  $4^{\circ}\text{C}$ . There is evidence that they are stable at higher temperatures, but no long-term controlled experiments have been reported. The inactivated vaccines must be stored at  $4^{\circ}\text{C}$ , and their shelf- life is usually given as 1 year.

No preservatives other than a protectant, such as sucrose and lactalbumin hydrolysate, are required for the freeze-dried preparation.

### 3. Vaccines based on biotechnology

#### 3.1. Vaccines available and their advantages

Currently, no recombinant vaccines for capripoxviruses are commercially available. However, a new generation of capripox vaccines is being developed that uses the capripoxvirus genome as a vector for the genes of other ruminant pathogens such as peste des petits ruminants (PPR) virus (Berhe *et al.*, 2003; Tuppurainen *et al.*, 2014).

#### 3.2. Special requirements for biotechnological vaccines, if any

Not applicable.

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**NB:** There are WOA Reference Laboratories for sheep pox and goat pox (please consult the WOA Web site: <https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).

Please contact the WOA Reference Laboratories for any further information on diagnostic tests, reagents and vaccines for sheep pox and goat pox

**NB:** FIRST ADOPTED IN 1989. MOST RECENT UPDATES ADOPTED IN 2024 (SECTION ON DIAGNOSTIC TECHNIQUES).