**Annex 15. Item 5.1. – Chapter 3.8.12. Sheep pox and goat pox**

2 MEETING OF THE BIOLOGICAL STANDARDS COMMISSION

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4

5 **CHAP T E R 3 . 8 . 1 2 .**

# 6 S H E EP P OX A N D GOA T POX

7 **SUMMARY**

1. *Sheep pox and goat pox are contagious, viral diseases of sheep and goats characterised by fever,*
2. *generalised papules or nodules, vesicles* (*rarely*), *internal lesions* (*particularly in the lungs*), *and death. Both*
3. *diseases are caused by strains of capripoxvirus, all of which can infect sheep and goats. Although most of*
4. *the strains examined cause more severe clinical disease in either sheep or goats, some strains have been*
5. *isolated that are equally pathogenic in both species.*
6. *Sheeppox virus* (*SPPV*) *and goatpox virus* (*GTPV*) *are the causative agents of sheep pox and goat pox, and*
7. *with lumpy skin disease virus* (*LSDV*) *make up the genus* Capripoxvirus *in the family* Poxviridae*. Sheep pox*
8. *and goat pox are endemic in Africa north of the Equator, the Middle East and Asia, while some parts of*
9. *Europe have experienced outbreaks recently. See WAHIS* [(*https://wahis.woah.org/#/home*](https://wahis.woah.org/%23/home)) *for recent*
10. *information on distribution at the country level. ~~Countries that reported outbreaks of the disease between~~*
11. *~~2010 and 2015 include Bulgaria, Chinese Taipei, Israel, Kazakhstan, Kyrgyzstan, Mongolia, Morocco,~~*
12. *~~Greece and Russia, with Greece, Israel and Russia having experienced recurring incidences.~~*
13. ***Identification of the agent:*** *Laboratory confirmation of capripoxvirus is most rapid using the polymerase*
14. *chain reaction* (*PCR*) *method in combination with a clinical history consistent with generalised capripoxvirus*
15. *infection. Isolation of the virus is possible as capripoxviruses will grow on tissue culture of ovine, caprine or*
16. *bovine origin, although field isolates may require up to 14 days to grow or require one or more additional*
17. *tissue culture passage*(*s*)*. The virus causes intracytoplasmic inclusions that can be clearly seen using*
18. *haematoxylin and eosin staining. The antigen can also be detected in tissue culture using specific sera and*
19. *immunoperoxidase or immunofluorescence techniques. Capripoxvirus antigen and inclusion bodies may be*
20. *seen in stained cryostat or paraffin sections of biopsy or post-mortem lesion material.*
21. *~~An antigen-detection enzyme-linked immunosorbent assay~~* ~~(~~*~~ELISA~~*~~)~~ *~~using a polyclonal detection serum~~*
22. *~~raised against a recombinant immunodominant antigen of capripoxvirus has been developed.~~*
23. ***Serological tests:*** *The virus neutralisation test is the most specific serological test. The indirect*
24. *immunofluorescence test is less specific due to cross-reactions with antibody to other poxviruses. Western*
25. *blotting using the reaction between the P32 antigen of capripoxvirus with test sera is both sensitive and*
26. *specific, but is expensive and difficult to carry out. An enzyme-linked immunosorbent assay* (*ELISA*) *has*
27. *been developed and validated to detect antibodies to capripoxviruses, however it cannot differentiate*
28. *between SPPV, GTPV and LSDV.*
29. *~~The use of this antigen, or other appropriate antigens expressed by a suitable vector, in an ELISA offers the~~*
30. *~~prospect of an acceptable and standardised serological test in the future.~~*
31. ***Requirements for vaccines:*** *Live and inactivated vaccines have been used for the control of*
32. *capripoxviruses. All strains of capripoxvirus so far examined share a major neutralisation site and some will*
33. *cross protect. Inactivated vaccines give, at best, only short-term immunity.*

## 41 A. INTRODUCTION

1. The *Capripoxvirus* genus, in the family *Poxviridae,* consists of three species – lumpy skin disease virus (LSDV), which
2. causes disease in cattle only (see Chapter 3.4.12), and sheeppox virus (SPPV) and goatpox virus (GT~~P~~PV), which cause
3. sheep pox and goat pox, respectively. Sheep pox and goat pox are characterised by disseminated cutaneous nodules and
4. up to 100% mortality in fully susceptible breeds of sheep and goats. In indigenous animals, generalised disease and
5. mortality are less common, although they are seen where disease has been absent from an area or village for a period of
6. time, when intensive husbandry methods are introduced, or in association with other disease agents, such as peste des
7. petits ruminants virus or foot and mouth disease virus. Sheep pox and goat pox are major constraints to the introduction
8. of exotic breeds of sheep and goats to endemic areas, and to the development of intensive livestock production.
9. Strains of SPPV and GTPV can pass between sheep and goats, although most cause more severe clinical disease in ~~only~~
10. ~~one~~ their homologous host species. SPPV and GTPV are transboundary diseases that regularly spread into adjacent, non-
11. endemic areas. Sheep pox and goat pox are endemic in Africa north of the Equator and parts of the Middle East and Asia
12. (see WAHIS for most up-to-date information on distribution: [https://wahis.woah.org/#/home](https://wahis.woah.org/%23/home)). Outbreaks have been
13. reported in non-endemic countries of Asia, Europe and the Middle East.
14. The incubation period of sheep pox and goat pox is between 8 and 13 days following contact between infected and
15. susceptible animals. It may be as short as 4 days following experimental infection by intradermal inoculation ~~or mechanica~~l
16. ~~transmission by insects~~. Some breeds of European sheep, such as Soay, may die of acute infection before the
17. development of skin lesions. In other breeds there is an initial rise in rectal temperature to above 40°C, followed in 2–5
18. days by the development of, at first, macules – small circumscribed areas of hyperaemia, which are most obvious on
19. unpigmented skin – and then of papules – hard swellings of between 0.5 and 1 cm in diameter – which may cover the body
20. or be restricted to the groin, axilla and perineum. Papules may be covered by fluid-filled vesicles, but this is rare. Some
21. researchers have distinguished between a vesicular and nodular form of sheep pox and goat pox (Zro *et al*., 2014b).
22. Within 24 hours of the appearance of generalised papules, affected animals develop rhinitis, conjunctivitis and enlargement
23. of all the superficial lymph nodes, in particular the prescapular lymph nodes. Papules on the eyelids cause blepharitis of
24. varying severity. As the papules on the mucous membranes of the eyes and nose ulcerate, so the discharge becomes
25. mucopurulent, and the mucosae of the mouth, anus, and prepuce or vagina become necrotic. Breathing may become
26. laboured and noisy due to pressure on the upper respiratory tract from the swollen retropharyngeal lymph nodes, due to
27. the developing lung lesions.
28. If the affected animal does not die in this acute phase of the disease, the papules start to become necrotic from ischaemic
29. necrosis following thrombi formation in the blood vessels at the base of the papule. In the following
30. 5–10 days the papules form scabs, which persist for up to 6 weeks, leaving small scars. The skin lesions are susceptible
31. to fly strike, and secondary pneumonia is common. Anorexia is not usual unless the mouth lesions physically interfere with
32. feeding. Abortion is rare.
33. On post-mortem examination of the acutely infected animal, the skin lesions are often less obvious than on the live animal.
34. The mucous membranes appear necrotic and all the body lymph nodes are enlarged and oedematous. Papules, which
35. may be ulcerated, can usually be found on the abomasal mucosa, and sometimes on the wall of the rumen and large
36. intestine, on the tongue, hard and soft palate, trachea and oesophagus. Pale areas of approximately 2 cm in diameter may
37. occasionally be seen on the surface of the kidney and liver, and have been reported to be present in the testicles. Numerous
38. hard lesions of up to 5 cm in diameter are commonly observed throughout the lungs, but particularly in the diaphragmatic
39. lobes.
40. The clinical signs and post-mortem lesions vary considerably with breed of host and strain of capripoxvirus. Indigenous
41. breeds are less susceptible and frequently show only a few lesions, which could be confused with insect bites or contagious
42. pustular dermatitis. However, lambs that have lost their maternally derived immunity, animals that have been kept isolated
43. and animals brought into endemic areas from isolated villages, particularly if they have been subjected to the stress of
44. moving long distances and mixing with other sheep and goats, and their pathogens, can often be seen with generalised
45. and sometimes fatal capripoxvirus infections. Invariably there is high mortality in unprotected imported breeds of sheep
46. and goats following capripoxvirus infection. Capripoxvirus is not infectious to humans.

## 88 B. DIAGNOSTIC TECHNIQUES

1. ***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 |
| **Identification of the agent(a)** | | | | | | |
| **Virus isolation** | + | ++ | + | +++ | + | – |
| **~~Antigen~~ ~~detection~~** | ~~++~~ | ~~++~~ | ~~++~~ | ~~++~~ | ~~++~~ | ~~–~~ |
| **IFAT** | + | + | + | ++ | + | – |
| **IHC** | + | + | + | ++ | + | – |
| **PCR** | ++ | +++ | ++ | +++ | ++ | – |
| **Detection of immune response** | | | | | | |
| **VNT** | ++ | ++ | ++ | ++ | ++ | ++ |
| **IFAT** | + | + | + | + | + | + |
| **ELISA** | ++ | ++ | ++ | ++ | ++ | ++ |

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

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

1. IFAT = indirect fluorescent antibody test; IHC = ; immunohistochemistry; PCR = polymerase chain reaction;
2. VNT = virus neutralisation; ELISA = enzyme-linked immunosorbent assay.
3. (a)~~A combination of agent identification methods applied on the same clinical sample is recommended.~~

#### 1. Identification of the agent

##### 1.1. Specimen collection and submission

1. Material for virus isolation and antigen detection should be collected by biopsy or at post-mortem from skin papules,
2. lung lesions or lymph nodes. Samples for virus isolation ~~and antigen-detection enzyme-linked immunosorbent assay~~
3. ~~(ELISA)~~ should be collected within the first week of the occurrence of clinical signs, before the development of
4. neutralising antibodies. Samples for genome detection by polymerase chain reaction (PCR) may be collected before
5. or after the development of neutralising antibody responses. Buffy coat from blood collected into EDTA (ethylene
6. diamine tetra-acetic acid) during the viraemic stage of capripoxvirus infection (before generalisation of lesions or
7. within 4 days of generalisation), can also be used for virus isolation.
8. Samples for histology should include tissue from the surrounding area and should be placed immediately following
9. collection into ten times the sample volume of 10% formalin or neutral buffered 10% formal saline. Tissues in formalin
10. have no special transportation requirements.
11. Blood samples, for virus isolation from the buffy coat, should be collected in tubes containing anticoagulant, placed
12. immediately on ice and processed as soon as possible. In practice, the blood samples may be kept at 4°C for up to
13. 2 days prior to processing, but should not be frozen or kept at ambient temperatures. Tissues and dry scabs for virus
14. isolation, antigen detection and genome detection should preferably be kept at 4°C, on ice or at –20°C. If it is
15. necessary to transport samples over long distances without refrigeration, the medium should contain 10% glycerol;
16. the samples should be of sufficient size that the transport medium does not penetrate the central part of the biopsy,
17. which should be used for virus isolation/detection.

##### 1.2. Virus isolation

1. Lesion material for virus isolation and antigen detection is homogenised. The following is an example of one technique
2. for homogenisation: The tissue is minced using sterile scissors and forceps, and then macerated in a steel ball bearing
3. mixer mill or ground with a sterile pestle in a mortar with sterile sand and an equal volume of sterile phosphate
4. buffered saline (PBS) or serum-free Modified Eagle’s Medium (MEM) containing sodium penicillin (1000 international
5. units [IU]/ml), streptomycin sulphate (1 mg/ml), mycostatin (100 IU/ml) or fungizone (2.5 µg/ml) and neomycin
6. (200 IU/ml). The homogenised suspension is freeze–thawed three times and then partially clarified by centrifugation
7. using a bench centrifuge at 600 ***g*** for 10 minutes. In cases where bacterial contamination of the sample is expected
8. (such as when virus is isolated from skin samples), the supernatant can be filtered through a 0.45 µm pore size filter
9. after the centrifugation step, however, the amount of virus in the supernatant might be reduced. Buffy coats may be
10. prepared from 5–8 ml unclotted blood by centrifugation at 600 ***g*** for 15 minutes; the buffy coat is carefully removed
11. into 5 ml of cold double-distilled water using a sterile Pasteur pipette. After 30 seconds, 5 ml of cold double-strength
12. growth medium is added and mixed. The mixture is centrifuged at 600 ***g*** for 15 minutes, the supernatant is discarded
13. and the cell pellet is suspended in 5 ml of growth medium, such as Glasgow’s modified Eagle’s medium (GMEM).
14. After centrifugation at 600 ***g*** for a further 15 minutes, the resulting pellet is suspended in 5 ml of fresh GMEM.
15. Alternatively, the buffy coat may be separated from a heparinised sample using a density gradient.
16. Capripoxvirus will grow in tissue culture of bovine, ovine or caprine origin, although primary or secondary cultures of
17. lamb testis (LT) or lamb kidney (LK) cells are considered to be the most susceptible. Care needs to be taken to ensure
18. they are not contaminated with viruses such as bovine viral diarrhoea virus~~, particularly those derived from a woo~~l
19. ~~sheep breed~~ (see chapter 1.1.9). Madin–Darby bovine kidney (MDBK) cells have been shown to be suitable for
20. capripoxvirus isolation (Fay *et al.,* 2020). The following is an example of an isolation technique: either 1 ml of buffy
21. coat cell suspension or 1 ml of clarified biopsy preparation supernatant is inoculated on to a 25 cm2 tissue culture
22. flask of appropriate cells at 90% ~~confluent LT or LK cells~~ confluence, and the supernatant is allowed to adsorb for 1
23. hour at 37°C. The culture is then washed with warm PBS and covered with 10 ml of a suitable medium, such as
24. GMEM, containing antibiotics and 2% fetal calf serum. If available, tissue culture tubes ~~containing LT or LK cells and~~
25. ~~a~~, flying cover-slips, or tissue culture microscope slides, ~~are~~ can also infected.
26. The flasks should be examined daily for 7–14 days for evidence of cytopathic effect (CPE). Contaminated flasks
27. should be discarded. Infected cells develop a characteristic CPE consisting of retraction of the cell membrane from
28. surrounding cells, and eventually rounding of cells and margination of the nuclear chromatin. At first only small areas
29. of CPE can be seen, sometimes as soon as 4 days after infection; over the following 4–6 days these expand to
30. involve the whole cell sheet. If no CPE is apparent by day 7, the culture should be freeze–thawed three times, and
31. clarified supernatant inoculated on to fresh ~~LT or LK~~ cell cultures. At the first sign of CPE in the flasks, or earlier if a
32. number of infected cover-slips are being used, a cover-slip should be removed, fixed in acetone and stained using
33. H&E. Eosinophilic intracytoplasmic inclusion bodies, which are variable in size but up to half the size of the nucleus
34. and surrounded by a clear halo, are indicative of poxvirus infection. Syncytia formation is not a feature of capripoxvirus
35. infection. If the CPE is due to capripoxvirus infection of the cell culture, it can be prevented or delayed by inclusion of
36. specific anti-capripoxvirus serum in the medium; this provides a presumptive identification of the agent. Some strains
37. of capripoxvirus have been adapted to grow on African green monkey kidney (Vero) cells, but these cells are not
38. recommended for primary isolation.

##### 1.3. Electron microscopy

1. The characteristic poxvirus virion can be visualised using a negative-staining preparation technique followed by
2. examination with an electron microscope. There are many different negative-staining protocols, an example is given
3. below:
4. Material from the original tissue suspension is prepared for transmission electron microscope examination, prior to
5. centrifugation, by floating a 400-mesh hexagon electron microscope grid, with piloform-carbon substrate activated by
6. glow discharge in pentylamine vapour, on to a drop of the suspension placed on parafilm or a wax plate. After 1
7. 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%
8. phosphotungstic acid, pH 7.2, for 10 seconds. The grid is drained using filter paper, air-dried and placed in the
9. electron microscope. The capripoxvirus virion is brick shaped, covered in short tubular elements and measures
10. approximately 290 × 270 nm. A host-cell-derived membrane may surround some of the virions, and as many as
11. possible should be examined to confirm their appearance (Kitching & Smale, 1986).
12. The virions of capripoxvirus are indistinguishable from those of orthopoxvirus, but, apart from *Vaccinia* virus, no
13. orthopoxvirus causes lesions in sheep and goats. However, capripoxvirus is distinguishable from the virions of
14. parapoxvirus, that cause contagious pustular dermatitis, as they are smaller, oval in shape, and each is covered in a
15. single continuous tubular element, which appears as striations over the virion.

##### 1.4. Histopathology

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

##### 1.5. Immunological methods

1. 1.5.1. Fluorescent antibody tests
2. Capripoxvirus antigen can also be identified on infected cover-slips or tissue culture slides using fluorescent
3. antibody tests. Cover-slips or slides should be washed and air-dried and fixed in cold acetone for 10 minutes.
4. The indirect test using immune sheep or goat sera is subject to high background colour and nonspecific
5. reactions. However, a direct conjugate can be prepared from sera from convalescent sheep or goats or from
6. rabbits hyperimmunised with purified *Capripoxvirus*. Uninfected tissue culture should be included as a
7. negative control because cross-reactions, due to antibodies to cell culture antigens, can cause problems. The
8. fluorescent antibody tissue section technique has also been used on cryostat-prepared slides.

##### 1.6. Nucleic acid recognition methods

1. Amplification methods for detection of ~~the viral DNA genome are specific to the genus~~ *Capripoxvirus* DNA are ~~and~~
2. both specific and sensitive ~~for detection~~ throughout the course of disease, including before and after the emergence
3. of antibody responses. These methods include conventional PCR, real-time PCR, and most recently loop-mediated
4. isothermal amplification (LAMP). Nucleic acid recognition methods can be used to detect the *Capripoxvirus* genome
5. in biopsy, swab or tissue culture samples.
6. 1.6.1. Conventional PCR methods
7. Several conventional PCR methods have been reported with varying specificity for capripoxviruses
8. in general, SPPV, or GTPV (Heine *et al.,* 1999; Ireland & Binepal, 1998; Zro *et al.,* 2014a). A
9. conventional PCR assay that differentiates GTPV and LSDV from SPPV has been described (Lamien
10. *et al.,* 2011a). Conventional PCR methods are particularly useful for obtaining sufficient genetic
11. material necessary for species identification by subsequent sequence and phylogenetic analysis (Le
12. Goff *et al.,* 2009).
13. The conventional gel-based PCR method described below is a simple, fast and sensitive method for
14. the detection of capripoxvirus genome in EDTA blood, semen or tissue culture samples (Tuppurainen
15. *et al.,* 2005).
16. Test procedure
17. The extraction method described below can be replaced using commercially available DNA extraction
18. kits.
19. i) Freeze and thaw 200 µl of blood in EDTA, semen or tissue culture supernatant and suspend in
20. 100 µl of lysis buffer containing 5 M guanidine thiocyanate, 50 mM potassium chloride, 10 mM
21. Tris/HCl (pH 8); and 0.5 ml Tween 20.
22. ii) Cut skin and other tissue samples into fine pieces using a sterile scalpel blade and forceps.
23. Grind with a pestle in a mortar. Suspend the tissue samples in 800 µl of the above mentioned
24. lysis buffer.
25. iii) Add 2 µl of proteinase K (20 mg/ml) to blood samples and 10 µl of proteinase K (20 mg/ml) to
26. tissue samples. Incubate at 56°C for 2 hours or overnight, followed by heating at 100°C for
27. 10 minutes. Add phenol:chloroform:isoamylalcohol (25:24:1 [v/v]) to the samples in a 1:1 ratio.
28. Vortex and incubate at room temperature for 10 minutes. Centrifuge the samples at 16,060 ***g***
29. for 15 minutes at 4°C. Carefully collect the upper, aqueous phase (up to 200 µl) and transfer
30. into a clean 2.0 ml tube. Add two volumes of ice cold ethanol (100%) and 1/10 volume of 3 M
31. sodium acetate (pH 5.3). Place the samples at –20°C for 1 hour. Centrifuge again at 16,060 ***g***
32. for 15 minutes at 4°C and discard the supernatant. Wash the pellets with ice cold 70% ethanol
33. (100 µl) and centrifuge at 16,060 ***g*** for 1 minute at 4°C. Discard the supernatant and dry the
34. pellets thoroughly. Suspend the pellets in 30 µl of nuclease-free water and store immediately at
35. –20°C (Tuppurainen *et al*., 2005). Alternatively a column-based extraction kit may be used.
36. iv) The primers for this PCR assay were developed from the gene encoding the viral attachment
37. protein. The size of the expected amplicon is 192 bp (Ireland & Binepal, 1998). The primers
38. have the following gene sequences:
39. Forward primer 5’-TCC-GAG-CTC-TTT-CCT-GAT-TTT-TCT-TAC-TAT-3’
40. Reverse primer 5’-TAT-GGT-ACC-TAA-ATT-ATA-TAC-GTA-AAT-AAC-3’.
41. v) DNA amplification is carried out in a final volume of 50 µl containing: 5 µl of 10 × PCR buffer,
42. 1.5 µl of MgCl2 (50 mM), 1 µl of dNTP (10 mM), 1 µl of forward primer, 1 µl of reverse primer,
43. 1 µl of DNA template (~10 ng), 0.5 µl of *Taq* DNA polymerase and 39 µl of nuclease-free water.
44. The volume of DNA template required may vary and the volume of nuclease-free water must
45. be adjusted to the final volume of 50 µl.
46. vi) Run the samples in a thermal cycler as follows: 2 minutes at 95°C; then 45 seconds at 95°C,
47. 50 seconds at 50°C and 1 minute at 72°C (34 cycles); 2 minutes at 72°C and hold at 4°C until
48. analysis.
49. vii) Mix 10 µl of each sample with loading dye and load onto a 1.5% agarose gel in TAE buffer
50. (Tris/acetate buffer containing EDTA). Load a parallel lane with a 100 bp DNA-marker ladder.
51. Electrophoretically separate the products using approximately 8–10 V/cm for 40–60 minutes
52. and visualise with a suitable DNA stain and transilluminator.
53. 1.6.2. Real-time PCR methods
54. Several highly sensitive and specific fluorescent detection-based real-time PCR methods have been
55. developed and validated (Balinsky *et al*., 2008; Bowden *et al.,* 2008; Das *et al.,* 2012; Stubbs *et al.,*
56. 2012). Each test detects a small conserved genetic locus within the capripoxvirus genome, but these
57. methods do not discriminate between SPPV, GTPV or LSDV. Real-time PCR methods for direct
58. capripoxvirus ~~genotyping~~ species differentiation without the need for gene sequencing have been
59. described (Gelaye *et al.,* 2013; Lamien *et al.,* 2011b; Wolff *et al.,* 2021).
60. The real-time PCR method described below is a rapid, sensitive and specific method for the detection
61. of DNA from SPPV, GTPV or LSDV. This assay will not differentiate between capripoxvirus species.
62. **DNA extraction from blood and tissue**
63. A number of DNA extraction kits are commercially available for the isolation of template DNA for real-
64. time PCR. Manufacturer’s instructions should always be consulted for guidance on the appropriate
65. method for the sample type being extracted. WOAH Reference Laboratories can be contacted for
66. advice on suitable commercial kits.
67. **Real-time PCR**
68. i) The real-time PCR method outlined below uses the primers and probe described by Bowden *et*
69. *al*. (2008). and further validated by Stubbs *et al.* (2012). Cycling conditions and reagent
70. concentrations can be altered to ensure optimal performance in individual laboratories.
71. ii) Forward and reverse primers should be prepared at concentrations of 20 µM. A minor grove
72. binder (MGB) TaqMan probe should be prepared at a concentration of 10 µM.
73. iii) Forward primer: 5’-AAA-ACG-GTA-TAT-GGA-ATA-GAG-TTG-GAA-3’
74. iv) Reverse primer: 5’-AAA-TGA-AAC-CAA-TGG-ATG-GGA-TA-3’
75. v) Probe: 5’-FAM-TGG-CTC-ATA-GAT-TTC-CT-MGB-3’
76. vi) Mastermix is prepared by combining 10 µl of 2 × real-time PCR mastermix with 0.4 µl of forward
77. primer, 0.4 µl of reverse primer, 0.5 µl of probe and 6.7 µl of RNase free water per reaction.
78. vii) Add 2 µl of extracted DNA to 18 µl of mastermix in a 96-well PCR plate or PCR strip and perform
79. real-time PCR according to the example given below or similar method:
80. viii) 95°C for 10 minutes followed by 45 cycles of 95°C for 15 seconds, 60°C for 60 seconds.
81. Fluorescence detection should be performed at the end of each cycle.
82. ix) Following completion of the real-time PCR, a cycle threshold (CT) should be set. Samples with
83. CT values less than 35 are considered positive. Samples with a CT value greater than 35 but
84. less than 45 are considered inconclusive and require further investigation. Samples which do
85. not yield a CT value, i.e. the amplification curve does not cross the threshold, are considered
86. negative.
87. 1.6.3. Isothermal genome amplification
88. Molecular tests using loop-mediated isothermal amplification (LAMP) to detect capripoxvirus
89. genomes are reported to provide sensitivity and specificity similar to real-time PCR with a simpler
90. method and at lower cost (Das *et al.,* 2012; Murray *et al.,* 2013). Field validation of the Das *et al.*
91. (2012) LAMP method has been further reported by (Omoga *et al.,* 2016) and a combination of this
92. universal capripoxvirus test with two additional LAMP assays was reported to show utility in
93. discriminating between GTPV and SPPV (Zhao *et al.,* 2014).

#### 2. Serological tests

##### 2.1. Virus neutralisation

1. A test serum can either be titrated against a constant titre of capripoxvirus (100 TCID50 [50% tissue culture infective
2. dose]) or a standard capripoxvirus strain can be titrated against a constant dilution of test serum in order to calculate
3. a neutralisation index. Because of the variable sensitivity of tissue culture to capripoxvirus, and the consequent
4. difficulty of ensuring the use of 100 TCID50, the neutralisation index is the preferred method, although it does require
5. a larger volume of test sera. The test is described using 96-well flat-bottomed tissue culture grade microtitre plates,
6. but it can be performed equally well in tissue culture tubes with the appropriate changes to the volumes used, although
7. it is more difficult to read an end-point in tubes. ~~The use of Vero cells in the virus neutralisation test has been reported~~
8. ~~to give more consistent results (Kitching & Taylor, 1985).~~
9. 2.1.1. Test procedure
10. i) Test sera including a negative and a positive control are diluted 1/5 in Eagle’s/HEPES (N-2-
11. hydroxyethylpiperazine, N-2-ethanesulphonic acid) and inactivated at 56°C for 30 minutes.
12. ii) Next, 50 µl of the first inactivated serum is added to columns 1 and 2, rows A to H of the
13. microtitre plate. The second serum is placed in columns 3 and 4, the third in columns 5 and 6,
14. the positive control serum is placed in columns 7 and 8, the negative control serum is placed in
15. columns 9 and 10, and 50 µl of Eagle’s/HEPES without serum is placed in columns 11 and 12
16. and to all wells of row H.
17. iii) A reference strain of capripoxvirus, usually a vaccine strain known to grow well in tissue culture,
18. with a titre of over log10 6 TCID50 per ml is diluted in Eagle’s/HEPES in bijoux bottles to give a
19. log dilution series of log10 5.0; 4.0; 3.5; 3.0; 2.5; 2.0; 1.5 TCID50 per ml (equivalent to log10 3.7;
20. 2.7; 2.2; 1.7; 1.2; 0.7; 0.2 TCID50 per 50 µl).
21. iv) Starting with row G and the most diluted virus preparation, 50 µl of virus is added to each well
22. in that row. This is repeated with each virus dilution, the highest titre virus dilution being placed
23. in row A.
24. v) The plates are covered and incubated for 1 hour at 37°C.
25. vi) ~~LT cells are~~ An appropriate cell suspension (such as MDBK cells) is prepared from pregrown
26. monolayers as a suspension of 105 cells/ml in Eagle’s medium containing antibiotics and 2%
27. fetal calf serum. Following incubation of the microtitre plates, 100 µl of cell suspension is added
28. to all the wells, except wells H11 and H12, which serve as control wells for the medium. The
29. remaining wells of row H are cell and serum toxicity controls.
30. vii) The microtitre plates are covered and incubated at 37°C for 9 days.
31. viii) Using an inverted microscope, the monolayers are examined daily starting at day 4 for evidence
32. of CPE. There should be no CPE in the cells of row H. Using the 0240 KSGP vaccine strain of
33. capripoxvirus, the final reading is taken on day 9, and the titre of virus in each duplicate titration
34. is calculated according to the Kärber method. If left longer, there is invariably a ‘breakthrough’
35. of virus in which virus that was at first neutralised appears to disassociate from the antibody.
36. ix) *Interpretation of the results:* The neutralisation index is the log titre difference between the titre
37. of the virus in the negative serum and in the test serum. An index of ≥1.5 is positive. The test
38. can be made more sensitive if serum from the same animal is examined before and after
39. infection. Because immunity to capripoxvirus is predominantly cell mediated, a negative result,
40. particularly following vaccination in which the response is necessarily mild, does not imply that
41. the animal from which the serum was taken is not protected.
42. ~~A constant-virus/varying-serum method has been described using serum dilutions in the range~~
43. ~~1/5 to 1/500 and fetal calf muscle cells. Because these cells have a lower sensitivity to~~
44. ~~capripoxvirus than LT cells, the problem of virus ‘breakthrough’ is overcome.~~

##### 2.2. Indirect fluorescent antibody test

1. Capripoxvirus-infected tissue culture grown on flying cover-slips or tissue culture microscope slides can be used for
2. the indirect fluorescent antibody test. Uninfected tissue culture control, and positive and negative control sera, should
3. be included in the test. The infected and control cultures are fixed in acetone at –20°C for 10 minutes and stored at
4. 4°C. Dilutions of test sera are made in PBS, starting at 1/5, and positives are identified using an anti-sheep gamma-
5. globulin conjugated with fluorescein isothiocyanate (Davies & Otema, 1978). Cross-reactions can occur with orf,
6. bovine papular stomatitis virus and perhaps other poxviruses.

##### 2.3. Western blot analysis

1. Western blotting of test sera against capripoxvirus-infected cell lysate provides a sensitive and specific system for
2. the detection of antibody to capripoxvirus structural proteins, although the test is expensive and difficult to carry out
3. (Chand *et al.*, 1994).

##### 2.4. Enzyme-linked immunosorbent assay

1. ~~No validated ELISA is available for the serological diagnosis of SPP or GTP.~~
2. Both in-house and commercial enzyme-linked immunosorbent assay (ELISAs) are available, but these tests cannot
3. discriminate between antibodies to different capripoxviruses (LSD or SPP/GTP).

## 346 C. REQUIREMENTS FOR VACCINES

347 **[THIS SECTION IS UNDER REVIEW IN THE 2024/2025 REVIEW CYCLE]**

#### 1. Background

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

1. A variety of attenuated live and inactivated capripoxvirus vaccines has been used to provide protection against
2. sheeppox and goatpox. All strains of capripoxvirus of ovine, caprine or bovine origin examined so far share a major
3. neutralising site, so that animals recovered from infection with one strain are resistant to infection with any other strain
4. (Capstick, 1961). Consequently, it is possible to use a single strain of capripoxvirus to protect both sheep and goats
5. against all field strains of virus, regardless of whether their origin was in Asia or Africa (Kitching *et al.*, 1986; Kitching
6. & Taylor, 1985). However, field evidence suggests some strains are quite host-specific and are used only in sheep
7. against SPPV and only in goat against GTPV.
8. A number of strains of capripoxvirus have had widespread use as live vaccines (Davies & Mbugwa, 1985), for
9. example the Romanian and RM-65 strains used mainly in sheep and the Mysore and Gorgan strains used in goats.
10. The real identity of the commonly used Kenyan sheep and goat pox vaccine virus (KSGP) 0240 was recently shown
11. to be actually LSDV (Tuppurainen *et al.,* 2014). Virus strain identity and attenuation properties must be ascertained
12. and taken into consideration when selecting vaccine strains for use in cattle, sheep and goats. The protective dose
13. depends on the vaccine strain used. Immunity in sheep and goats against capripoxvirus following vaccination with
14. the 0240 strain lasts over a year and the Romanian strain gave protection for at least 30 months.
15. Killed vaccines produced from tissue culture contain only the intracellular mature virion form of the virus, and lack the
16. less robust but biologically crucial extracellular enveloped virion form. As a result, the vaccine does not stimulate
17. immunity against the extracellular enveloped virion, resulting in poor protection. Killed capripoxvirus vaccines provide,
18. at best, only temporary protection.

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

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

##### 2.1. Characteristics of the seed

1. 2.1.1. Biological characteristics
2. A strain of capripoxvirus used for vaccine production must be accompanied by a history describing
3. its origin and tissue culture or animal passage. It must be safe to use in all breeds of sheep and goats
4. for which it is intended, including pregnant and young animals. It must be non-transmissible, remain
5. attenuated after further tissue culture passage, and provide complete protection against challenge
6. with virulent field strains for a minimum of 1 year. A quantity of master seed vaccine virus should be
7. prepared and stored in order to provide a consistent working seed for regular vaccine production.
8. 2.1.2. Quality criteria (sterility, purity, freedom from extraneous agents)
9. Each master seed must be tested to ensure its identity and shown to be free from adventitious
10. viruses, in particular pestiviruses, such as border disease and bovine viral diarrhoea virus, and free
11. from contamination with bacteria, fungi and/or mycoplasmas. The general procedures for sterility or
12. purity tests are described in chapter 1.1.9. The master seed must also be safe and produce no clinical
13. reaction in all breeds of sheep or goats when given by the recommended route and stimulate
14. complete immunity to capripoxvirus in all breeds of sheep and goats for at least 1 year. The necessary
15. safety and potency tests are described in Section C.2.2.4 *Final product batch tests*.

##### 2.2. Method of manufacture

1. The method of manufacture should be documented as the Outline of Production.
2. 2.2.1. Procedure
3. Vaccine seed should be lyophilised and stored in 2 ml vials at –20°C. It may be stored wet at –20°C,
4. but when wet, is more stable at –70°C or lower. The virus should be cultured in primary or secondary
5. LT or LK cells of wool sheep origin for maximum yield. Vero cells may also be used with suitably
6. adapted strains.
7. Vaccine batches are produced on fresh monolayers of secondary LT or primary LK cells. A vial of
8. seed virus is reconstituted with GMEM or another appropriate medium and inoculated on to an LT or
9. LK monolayer that has been previously washed with warm PBS, and allowed to adsorb for 15 minutes
10. at 37°C before being overlaid with additional GMEM. After 4–6 days, there will be extensive (80–
11. 90%) CPE. The culture should be examined for any evidence of nonspecific CPE, medium cloudiness
12. or change in medium pH. The culture is freeze–thawed three times, the suspension removed and
13. centrifuged at 600 ***g*** for 20 minutes. A second passage may be required to produce sufficient virus
14. for a production batch. Live vaccine may be produced on roller bottles.
15. The procedure is repeated and the harvests from individually numbered flasks are each mixed
16. separately with an equal volume of sterile and chilled 5% lactalbumin hydrolysate and 10% sucrose,
17. and transferred to individually numbered bottles for storage at –20°C. Prior to storage, 0.2 ml is
18. removed from each bottle for sterility control. An additional 0.2 ml is removed for virus titration; 2 ml
19. pools composed of 0.2 ml samples taken from ten bottles are used. A written record of all the
20. procedures must be kept for all vaccine batches.
21. Inactivated vaccines are produced, usually from unattenuated field strains of capripoxvirus, grown in
22. tissue culture as described above, inactivated with 0.03% formaldehyde, and mixed with an equal
23. volume of alhydrogel as adjuvant. Formaldehyde is no longer considered to be a suitable inactivant
24. for certain viral vaccines because its mode of action cannot be guaranteed to be totally effective in
25. inactivating all the live virus. This has not been fully investigated for capripoxvirus.
26. 2.2.2. Requirements for substrate and media
27. The specification and source of all ingredients used in the manufacturing procedure should be
28. documented and the freedom from extraneous agents: bacteria, fungi, mycoplasma and any other
29. viruses should be tested. The detailed testing procedure is described in the chapter 1.1.9. The use
30. of antibiotics must meet the requirements of the licensing authority.
31. 2.2.3. In-process controls
32. i) Cells
33. Cells should be obtained from the testis or kidney of a healthy young lamb from a scrapie-free flock
34. of a wool sheep breed. During cultivation, cells must be observed for any evidence of CPE, and for
35. normal morphology (predominantly fibroblastic). They can usually be passaged successfully up to
36. ten times. When used for vaccine production, uninfected control cultures should be grown in parallel
37. and maintained for at least three additional passages for further observation. They should be checked
38. for the presence of noncytopathic strains of bovine virus diarrhoea or border disease viruses by
39. immunofluorescence or immunoperoxidase techniques. If possible, cells should be prepared and
40. screened prior to vaccine production and stocked in 1–2 ml aliquots containing 2 × 107 cells/ml in
41. sterile 10% DMSO (dimethyl sulphoxide) and 90% FBS (fetal bovine serum) solution stored in liquid
42. nitrogen.
43. ii) Serum
44. Bovine serum used in the growth or maintenance medium must be free from transmissible spongiform
45. encephalopathies (TSEs) and antibody to capripoxvirus, and tested for contamination with pestivirus
46. or any other viruses, extraneous bacteria, mycoplasma or fungi.
47. iii) Medium
48. Medium must be tested free from contamination with pestivirus or any other viruses, extraneous
49. bacteria, mycoplasma or fungi.
50. iv) Virus
51. Seed virus and final vaccine must be titrated in tissue culture tubes or microtitre plates. Vaccine
52. samples must be examined for the presence of adventitious viruses including cytopathic and
53. noncytopathic strains of pestivirus, and should be mixed with a high-titre capripoxvirus-immune
54. serum that has tested negative for antibody to pestivirus to prevent the vaccine virus itself interfering
55. with the test. The vaccine bulk can be held at –20°C or below until all sterility tests and titrations have
56. been completed, at which time it should be freeze-dried in 1 ml aliquots in vials sufficient for 100
57. doses. The vaccine harvest diluted with lactalbumin hydrolysate and sucrose should have a minimum
58. titre log10 4.5 TCID50 per ml after freeze-drying, equivalent to a field dose of log10 2.5 TCID50. A further
59. titration is carried out on five randomly chosen vials of the freeze-dried preparation to confirm the
60. titre.
61. 2.2.4. Final product batch tests
62. i) Sterility/purity
63. Tests for sterility and freedom from contamination of biological materials intended for veterinary use
64. may be found in chapter 1.1.9.
65. ii) Safety
66. The safety studies should be demonstrated by statistically valid vaccination studies using
67. seronegative young sheep and goats of known susceptibility to capripox virus. The procedure
68. described is suitable for vaccine strains such as 0240 that are equally immunogenic in both sheep
69. and goats. The choice of target animal should be adapted for strains with a more restricted host
70. preference.
71. iii) Potency
72. Potency tests must be undertaken if the minimum immunising dose of the virus strain is not known.
73. This is usually carried out by comparing the titre of a virulent challenge virus on the flanks of
74. vaccinated and control animals. Following vaccination, the flanks of at least three animals and three
75. controls are shaved of wool or hair. Log10 dilutions of the challenge virus are prepared in sterile PBS
76. and six dilutions are inoculated intradermally (0.1 ml per inoculum) along the length of the flank; four
77. replicates of each dilution are inoculated down the flank. An oedematous swelling will develop at
78. possibly all 24 inoculation sites on the control animals, although preferably there will be little or no
79. reaction at the four sites of the most dilute inocula. The vaccinated animals should develop an initial
80. hypersensitivity reaction at sites of inoculation within 24 hours, which should quickly subside. Small
81. areas of necrosis may develop at the inoculation site of the most concentrated challenge virus. The
82. macule/papule is measured at between 8 and 10 days post-challenge. The titre of the challenge virus
83. is calculated for the vaccinated and control animals; a difference of log10 titre > 2.5 is taken as
84. evidence of protection.

##### 2.3. Requirements for authorisation

1. 2.3.1. Safety requirements
2. i) Target and non-target animal safety
3. The vaccine must be safe to use in all breeds of sheep and goats for which it is intended, including
4. young and pregnant animals. It must also be non-transmissible, remain attenuated after further tissue
5. culture passage.
6. Safety tests should be carried out on the final product of each batch as described in Section C.2.2.4.
7. The safety of the vaccine in non-target animals must have been demonstrated using mice and
8. guinea-pigs as described in Section C.2.2.4. There should be no evidence of pathology caused by
9. the vaccine.
10. ii) Reversion-to-virulence for attenuated/live vaccines
11. The selected final vaccine should not revert to virulence during a further passages in target animals.
12. iii) Environmental consideration
13. Attenuated vaccine should not be able to perpetuate autonomously in cattle, sheep or goat
14. populations. Vaccines using the 0240 strain should not be used in *Bos taurus* breeds. Strains of
15. capripoxvirus are not a hazard to human health. There are no precautions other than those described
16. above for sterility and freedom from adventitious agents.
17. 2.3.2. Efficacy requirements
18. i) For animal production
19. The efficacy of the vaccine must be demonstrated in vaccination challenge experiment under
20. laboratory conditions. As described in Section C.2.2.4.
21. Once the potency of the particular strain being used for vaccine production has been determined in
22. terms of minimum dose required to provide immunity, it is not necessary to repeat this on the final
23. product of each batch, provided the titre of virus present has been ascertained.
24. ii) For control and eradication
25. Vaccination is the only effective way to control the sheep pox and goat pox outbreaks in endemic
26. countries. Unfortunately, currently no marker vaccines allowing the differentiation of infected from
27. vaccinated animals are available.
28. Immunity to virulent field virus following vaccination of sheep or goats with the 0240 strain lasts over
29. 1 year, and protection against generalised infection following intradermal challenge lasts at least
30. 3 years and is effective lifelong. The duration of immunity produced by other vaccine strains should
31. be ascertained in both sheep and goats by undertaking controlled trials in an environment in which
32. there is no possibility of field strains of capripoxvirus confusing the results. The inactivated vaccines
33. provide immunity for less than 1 year, and for the reasons given at the beginning of this section, may
34. not give immunity to the form of capripoxvirus usually associated with natural transmission.
35. 2.3.3. Stability
36. All vaccines are initially given a shelf-life of 24 months before expiry. Real-time stability studies are
37. then conducted to confirm the appropriateness of the expiry date. Multiple batches of the vaccine
38. should be re-titrated periodically throughout the shelf-life to determine the vaccine variability.
39. Properly freeze-dried preparations of capripox vaccine, particularly those that include a protectant,
40. such as sucrose and lactalbumin hydrolysate, are stable for over 25 years when stored at –20°C and
41. for 2–4 years when stored at 4°C. There is evidence that they are stable at higher temperatures, but
42. no long-term controlled experiments have been reported. The inactivated vaccines must be stored at
43. 4°C, and their shelf- life is usually given as 1 year.
44. No preservatives other than a protectant, such as sucrose and lactalbumin hydrolysate, are required
45. for the freeze-dried preparation.

#### 3. Vaccines based on biotechnology

##### 3.1. Vaccines available and their advantages

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

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

1. Not applicable.

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589 \*

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1. **NB:** There are WOAH Reference Laboratories for sheep pox and goat pox (please consult the WOAH Web site:
2. <https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).
3. Please contact the WOAH Reference Laboratories for any further information on
4. diagnostic tests, reagents and vaccines for sheep pox and goat pox
5. **NB**: First adopted in 1989. Most recent updates adopted in 2017.