SECTION 3.5.

CAMELIDAE

CHAPTER 3.5.1.

CAMELPOX

SUMMARY

Camelpox is a wide-spread infectious viral disease of Old World camelids. New World camelids are also susceptible. It occurs throughout the camel-breeding areas of Africa, north of the equator, the Middle East and Asia, and has an important economic impact through loss of production and sometimes death. Camelpox does not occur in the feral camel population of Australia. Camelpox virus *belongs to the family* Poxviridae*, subfamily* Chordopoxvirinae*, genus* Orthopoxvirus*. The disease is characterised by fever, local or generalised pox lesions on the skin and in the mucous membranes of the mouth and respiratory tract. The clinical manifestations range from inapparent infection to mild, moderate and, less commonly, severe systemic infection and death. The disease occurs more frequently and more severely in young animals and pregnant females. Transmission is by either direct contact between infected and susceptible animals or indirect infection via a contaminated environment. The role of insects in transmission has been suspected because the disease is often observed after rainfall. Camelpox virus is very host specific and does not infect other animals. Zoonotic camelpox virus infection in humans associated with outbreaks in dromedary camels* (Camelus dromedarius) *was described in the north-eastern region of India during 2009. This was a single incident illustrating that camelpox is of limited public health importance.*

Detection of the agent: *The presumptive diagnosis of camelpox infection is based on clinical signs. However, infections of camels with contagious ecthyma* (*orf*), *papilloma virus and reaction to insect bites are considered differential diagnoses in the early clinical stages and in mild cases of camelpox. Several diagnostic methods are available and, where possible, more than one should be used to make a confirmatory diagnosis of disease.*

The preferred method of laboratory confirmation of camelpox is by the use of polymerase chain reaction (*PCR*) *methods. Alternatively the characteristic, brick-shaped orthopoxvirions can be demonstrated in skin lesions, scabs or tissue samples using transmission electron microscopy* (*TEM*). *Camelpox virus is distinct from the ovoid-shaped parapox virus, the aetiological agent of the principle differential diagnosis: camel orf. However, both viruses may be seen simultaneously by TEM as dual infections.*

Camelpox can be confirmed by demonstration of the camelpox antigen in scabs and pock lesions in tissues by immunohistochemistry. It is a relatively simple method that can be performed in laboratories where TEM is not available. In addition, the paraffin-embedded samples can be stored for a long period of time, enabling future epidemiological, retrospective studies.

Camelpox virus may be propagated on the chorioallantoic membrane (*CAM*) *of embryonated chicken eggs. After 5 days, characteristic lesions can be observed on the CAM. Camelpox virus shows typical cytopathic effect on a wide variety of cell cultures. Intracytoplasmic eosinophilic inclusion bodies, characteristic of poxvirus infection, may be demonstrated in infected cells using haematoxylin and eosin staining. The presence of viral nucleic acid may be confirmed by PCR, and different strains of camelpox virus may be identified using DNA restriction enzyme analysis. An antigen-capture*

enzyme-linked immunosorbent assay (*ELISA*) *for the detection of camelpox virus has been described.*

Serological tests: *A wide range of serological tests is available to identify camelpox, including virus neutralisation and ELISA.*

Requirements for vaccines: *Both attenuated and inactivated vaccines are commercially available. Vaccination with live attenuated vaccine provides protection for at least 6 years and with inactivated vaccine for 12 months.*

A. INTRODUCTION

Camelpox occurs in almost every country in which camel husbandry is practised apart from the introduced dromedary camel in Australia and tylopods (llama and related species) in South America. Outbreaks have been reported in the Middle East, Asia and Africa (Mayer & Czerny, 1990; Wernery *et al*., 1997b). The disease is endemic in many countries and a pattern of sporadic outbreaks occurs with a rise in the seasonal incidence usually during the rainy season. For up-to-date information, consult OIE WAHIS interface[1]).

Camelpox is caused by *Camelpox virus*, which belongs to the genus *Orthopoxvirus* within the family *Poxviridae.* Based on sequence analysis, it has been determined that the camelpox virus is the most closely related to variola virus, the aetiological agent for smallpox. Camels have been successfully vaccinated against camelpox with vaccinia virus strains. The average size of the virion is 265–295 nm. Orthopoxviruses are enveloped, brick-shaped and the outer membrane is covered with irregularly arranged tubular proteins. A virion consists of an envelope, outer membrane, two lateral bodies and a core. The double-stranded DNA genome is approximately 206 kbp. Virus replicates in the cytoplasm of the host cell, in so-called inclusion bodies. Camelpox virus haemagglutinates chicken erythrocytes, but the haemagglutination may be poor (Davies *et al*., 1975). Camelpox virus is ether resistant and chloroform sensitive (Davies *et al*., 1975; Tantawi *et al*., 1974). The virus is sensitive to pH 3–5 and pH 8.5–10 (Davies *et al*., 1975). Poxviruses are susceptible to various disinfectants including 1% sodium hypochlorite, 1% sodium hydroxide, 1% peracetic acid, 0.5–1% formalin and 0.5% quaternary ammonium compounds. The virus can be destroyed by autoclaving or boiling for 10 minutes and is killed by ultraviolet rays (245 nm wave length) in a few minutes (Coetzer, 2004).

The incubation period is usually 9–13 days (varying between 3 and 15 days). Clinical manifestations of camelpox range from inapparent and mild local infections, confined to the skin, to moderate and severe systemic infections, possibly reflecting differences between the strains of camelpox or differences in the immune status of the animals (Wernery *et al.,* 2014). The disease is characterised by fever, enlarged lymph nodes and skin lesions. Skin lesions appear 1–3 days after the onset of fever, starting as erythematous macules, developing into papules and vesicles, and later turning into pustules. Crusts develop on the ruptured pustules. These lesions first appear on the head, eyelids, nostrils and the margins of the ears. In severe cases the whole head may be swollen. Later, skin lesions may extend to the neck, limbs, genitalia, mammary glands and perineum. In the generalised form, pox lesions may cover the entire body. Skin lesions may take up to 4–6 weeks to heal. In the systemic form of the disease, pox lesions can be found in the mucous membranes of the mouth and respiratory tract (Kritz, 1982; Wernery *et al.,* 2014).

The animals may show salivation, lacrimation and a mucopurulent nasal discharge. Diarrhoea and anorexia may occur in the systemic form of the disease. Pregnant females may abort. Death is usually caused by secondary infections and septicaemia (Wernery *et al.,* 2014).

Histopathological examination of the early skin nodules reveals characteristic cytoplasmic swelling, vacuolation and ballooning of the keratinocytes of the outer stratum spinosum. The rupture of these cells produces vesicles and localised oedema. Perivascular infiltration of mononuclear cells and variable infiltration of neutrophils and eosinophils occurs. Marked epithelial hyperplasia may occur in the borders of the skin lesions (Yager *et al*., 1991).

There are only a few detailed pathological descriptions of internal camelpox lesions. The lesions observed on post-mortem examination of camels that die following severe infection with camelpox are multiple pox-like lesions on the mucous membranes of the mouth and respiratory tract. The size of the lesions in the lungs may vary in diameter between 0.5 and 1.3 cm, occasionally up to 4–5 cm. Smaller lesions may have a haemorrhagic centre. The lung lesions are characterised by hydropic degeneration, proliferation of bronchial epithelial cells, and infiltration of the

1 http://www.oie.int/en/animal-health-in-the-world/the-world-animal-health-information-system/the-world-animal-health-information-system/

affected areas by macrophages, necrosis and fibrosis (Kinne *et al*., 1998; Pfeffer *et al*., 1998a; Wernery *et al*., 1997a). Pox lesions are also observed in the mucosa of the trachea and retina of the eye causing blindness.

The morbidity rate of camelpox is variable and depends on whether the virus is circulating in the herd. Serological surveys taken in several countries reveal a high prevalence of antibodies to camelpox (Wernery *et al.,* 2014). The incidence of disease is higher in males than females, and the mortality rate is greater in young animals than in adults (Kritz, 1982). The mortality rate in adult animals is between 5% and 28% and in young animals between 25% and 100% (Mayer & Czerny, 1990).

Transmission is by either direct contact between infected and susceptible animals or indirect infection via a contaminated environment. The infection usually occurs by inhalation or through skin abrasions. Virus is secreted in milk, saliva, and ocular and nasal discharges. Dried scabs shed from the pox lesions may contain live virus for at least 4 months and contaminate the environment. The role of an arthropod vector in the transmission of the disease has been suspected. Camelpox virus has been detected by transmission electron microscopy (TEM) and virus isolation from the camel tick, *Hyalomma dromedarii*, collected from animals infected with camelpox virus. The increased density of the tick population during the rainy season may be responsible for the spread of the disease (Wernery *et al*., 1997a). However, other potential vectors may be involved, such as biting flies and mosquitoes.

Different strains of camelpox virus may show some variation in their virulence (Wernery *et al.,* 2014). Restriction enzyme analysis of viral DNA allows isolates to be compared. However, no major differences from the vaccine strain have so far been demonstrated (Wernery *et al*., 1997a).

Immunity against camelpox is both humoral and cell mediated. The relative importance of these two mechanisms is not fully understood, but it is believed that circulating antibodies do not reflect the immune status of the animal (Wernery *et al.,* 2014). Life-long immunity follows after natural infection. Live, attenuated vaccine provides protection against the disease for at least 6 years, probably longer (Wernery & Zachariah, 1999). Inactivated vaccine provides protection for 1 year only.

The camelpox virus is very host specific and does not infect other animal species, including cattle, sheep and goats. Several cases in humans have been described (Kritz, 1982); the most recent from India (Bera *et al.*, 2011) with clinical manifestations such as papules, vesicles, ulceration and finally scabs on fingers and hands. However, such cases and even milder human infections seem to be rare illustrating that camelpox is of limited public health importance. Camelpox virus should be handled with appropriate biosafety and biocontainment measures as determined by biorisk analysis (see Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*).

B. DIAGNOSTIC TECHNIQUES

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

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent[2]						
TEM	–	–	–	+++	–	–
Virus isolation in cell culture	–	–	–	+++	–	–
Virus isolation on CAM	–	–	–	+++	–	–
Immuno-histochemistry	–	–	–	+++	–	–
PCR	–	–	–	+++	–	–
Real-time PCR	–	–	–	+++	–	–

2 A combination of agent identification methods applied on the same clinical sample is recommended.

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

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
TEM: = Transmission electron microscopy; CAM = chorioallantoic membrane of embryonated chicken eggs;
PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay.

During the viraemic stage of the disease (within the first week of the occurrence of clinical signs) camelpox virus can be isolated in cell culture from heparinised blood samples, or viral DNA can be detected by the polymerase chain reaction (PCR) from blood in EDTA (ethylene diamine tetra-acetic acid). The blood samples should be collected in a sterile manner by venepuncture. Blood samples, with anticoagulant for virus isolation from the buffy coat, should be placed immediately on ice and processed as soon as possible. In practice, the samples can be kept at 4°C for up to 2 days prior to processing, but should not be frozen or kept at ambient temperatures.

Blood obtained for serum samples should be collected in plain tubes with no anticoagulant. The blood tubes should be left to stand at room temperature for 4–8 hours until the clot begins to contract, after which the blood is centrifuged at 1000 ***g*** for 10–15 minutes. Separated serum can be collected with a pipette and held at 4°C for a short period of time or stored at –20°C.

A minimum of 2 g of tissue from skin biopsies and organs should be collected for virus isolation and histopathology. Scabs from different locations of the body are also suitable for virus isolation and histopathology. They should be transported without additives in plain tubes. For the PCR, approximately 30–50 mg of tissue sample should be placed in a cryotube or similar container, kept at 4°C for transportation and stored at –20°C until processed. Tissue samples collected for virus isolation should be placed in a virus transport medium, such as Tris-buffered tryptose broth or minimal essential medium (MEM) without fetal calf serum (FCS), kept at 4°C for transportation and stored at –80°C until processed. Material for histology should be placed immediately after collection into ten times the sample volume of 10% formalin. The size of the samples should not exceed 0.5 cm × 1–2 cm. Samples in formalin can be transported at room temperature.

1. Detection of the agent

1.1. Transmission electron microscopy

TEM is a rapid method to demonstrate camelpox virus in scabs or tissue samples. However, a relatively high concentration of virus in the sample is required for positive diagnosis and camelpox virus cannot be differentiated from other *Orthopoxvirus* species. However, currently, TEM is the fastest method for distinguishing clinical cases of camelpox and orf respectively, although the poxvirus infections can be differentiated by serological techniques and by PCR (Mayer & Czerny, 1990).

1.1.1. Sample preparation

The size of a sample should be at least 30–50 mg. Mince the scabs or tissue sample with a disposable blade or sterile scissors and forceps. Grind the sample in a five-fold volume of phosphate-buffered saline (PBS) with antibiotics (such as 10^5 International Units [IU] penicillin and 10 mg streptomycin per ml) using a mortar and pestle with sterile sand. Transfer the sample into a centrifuge tube and freeze and thaw two to three times to release the virus from the cells. Vortex the samples while thawing. Place the tubes on ice and sonicate once for 30 seconds at 80 Hz. Centrifuge at 1000 ***g*** for 10 minutes to remove the gross particles and collect the supernatant (Pfeffer *et al.*, 1996; 1998b).

1.1.2. Test procedure

Place 10 µl of above-mentioned supernatant on poly-L-lysine-covered grids and incubate at room temperature for 5 minutes. Remove the fluid with a chromatography filter paper. Add one drop of 2% phosphotungstic acid (diluted in sterile water and pH adjusted to 7.2 with NaOH) to the grid,

incubate at room temperature for 5 minutes and air dry. Examine the grid by TEM (Pfeffer *et al*., 1996; 1998b).

Camelpox virus has a typical brick-shaped appearance with irregularly arranged, tubular surface proteins. Parapoxviruses are slightly smaller, ovoid-shaped and the surface proteins are regularly arranged.

1.2. Virus isolation in cell cultures

Camelpox virus can be propagated in a large variety of cell lines including Vero, MA-104 and MS monkey kidney, baby hamster kidney (BHK), and Dubai camel skin (Dubca) as well as in primary cell cultures (lamb testis, lamb kidney, camel embryonic kidney, calf kidney, and chicken embryo fibroblast) (Davies *et al*., 1975; Tantawi *et al*., 1974).

The samples are prepared for virus isolation as described above in Section B.1.1.1.

1.2.1. Test procedure

Incubate 400 µl of the supernatant for 1 hour at room temperature and then overnight at 4°C. Filter the supernatant through a 0.45 µm filter and inoculate into a 25 cm^2 flask of confluent cells. Flush the filter with 0.5 ml of the maintenance medium used in the cell culture and incubate the flasks at 37°C for 1 hour. Add 6–7 ml of fresh medium into the flask and continue the incubation for about 5–7 days. If there is any reason to suspect fungal contamination, the contaminated medium must be discarded and 5 µg/ml of amphotericin B added to a new medium. The flasks must be monitored daily for 5–7 days.

Characteristic, plaque-type cytopathic effect (CPE) showing foci of rounded cells, cell detachment, giant cell formation and syncytia may appear as soon as 24 hours post-inoculation. Syncytia may contain up to 20–25 nuclei (Tantawi *et al*., 1974). The growth of camelpox virus in a cell culture can be confirmed by TEM, PCR or antigen-capture enzyme-linked immunosorbent assay (ELISA) (Johann & Czerny, 1993).

1.3. Virus isolation on chorioallantoic membrane of embryonated chicken eggs

Camelpox virus can be isolated on the chorioallantoic membrane (CAM) of 11- to 13-day-old embryonating chicken eggs. The eggs should be incubated at 37°C and after 5 days the eggs containing living embryos are opened and the CAM examined for the presence of characteristic pock lesions: dense, greyish-white pocks. Camelpox virus does not cause death in inoculated embryonated chicken eggs. The maximum temperature for the formation of pock lesions is 38.5°C. If the eggs are incubated at 34.5°C, the pocks are flatter and a haemorrhagic centre may develop (Tantawi *et al*., 1974).

1.4. Immunohistochemistry

Immunohistochemistry for the detection of camelpox viral antigen is a relatively fast method and can be used instead of electron microscopy to establish a tentative diagnosis (Nothelfer *et al*., 1995). Almost any polyclonal antibody against vaccinia virus is likely to produce reasonable results in this test because of the antigenic relatedness between vaccinia and camelpox viruses (Nothelfer *et al*., 1995).

1.4.1. Test procedure

The following procedure for immunohistochemistry is described by Kinne *et al.* (1998) and Pfeffer *et al.* (1998b). The entire skin pustule should be collected for the immunohistochemical examination. Fix the tissue in 10% formalin, dehydrate through graded alcohols and embed in paraffin wax according to standard histopathological procedures. Cut approximately 3 µm sections and place on glass slides. Treat the deparaffinised and dehydrated sections with 3% H_2O_2, prepared in distilled water, for 5 minutes and wash with PBS. Incubate the slides for 60 minutes at 37°C with anti-vaccinia virus monoclonal antibody 5B4[3], diluted 1/500. Remove the monoclonal antibody by washing twice with cold PBS. Incubate the slides for 30 minutes with anti-mouse antibodies labelled with biotin. Wash with PBS for 5 minutes and incubate with streptavidin-peroxidase for 30 minutes. Wash again with PBS for 5 minutes and add

3 Monoclonal antibody 5B4 is available commercially. For further information, please contact the OIE Reference Laboratory (see Table in Part 4 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list).

diaminobenzidine as chromogen for 10 minutes. Examine the slides under the microscope using ×200–400 magnification for any brown-labelled pox antigen.

1.5. Polymerase chain reaction

The PCR is a fast and sensitive method for the detection of orthopoxviral DNA. Several gel-based PCR methods have been described for the detection of camelpox viral DNA (Balamurugan *et al*., 2009; Meyer *et al*., 1994; 1997; Ropp *et al*., 1995). A generic PCR assay, described by Meyer *et al.* (1994), allows the detection and differentiation of species of the genus *Orthopoxvirus* because of the size differences of the amplicons. Using the primer pair: 5'-AAT-ACA-AGG-AGG-ATC-T-3' and 5'-CTT-AAC-TTT-TTC-TTT-CTC-3', the gene sequence encoding the A-type inclusion protein (ATIP) will be amplified. The size of the PCR product, specific for the camelpox virus, is 881 bp.

1.5.1. Test procedure for gel-based PCR

Suspend a small aliquot of crusted scabs in 90 µl of lysis solution (50 mM Tris/HCl, pH 8.0, 100 mM Na_2 EDTA, 100 mM NaCl, 1% sodium dodecyl sulphate) and add 10 µl of proteinase K (20 mg/ml). Digest the sample for 10 minutes at 37°C prior to the disruption of the scab or tissue with a microfuge tube pestle. Add another 350 µl lysis solution and 50 µl of proteinase K, mix gently and incubate for 3 hours at 37°C. Extract the lysed suspension with an equal volume of phenol/chloroform/isoamyl alcohol (25/24/1) and centrifuge at 8000 ***g*** at 4°C for 1 minute. Collect the upper aqueous phase and mix it again with an equal volume of phenol/chloroform/isoamyl alcohol (25/24/1). Centrifuge at 8000 ***g*** at 4°C for 1 minute and transfer the upper, aqueous phase to a new tube. Precipitate the DNA by adding 1/10 volume of 3 M sodium acetate and two volumes of ice-cold absolute ethanol. Place the mixture at –70°C for 30 minutes or –20°C overnight. Centrifuge at 15,000 ***g*** for 5 minutes at 4°C. Discard the supernatant and wash the pellet with 0.5 ml of 70% ethanol. Centrifuge at 15,000 ***g*** for 5 minutes. Discard the supernatant and air-dry the pellets. Resuspend the pellets in 10 µl of nuclease-free water. Alternatively, DNA can be extracted using commercially available DNA extraction kits.

DNA amplification is carried out in a final volume of 50 µl containing 2 µl of each dNTP (10 mM), 5 µl of 10 × PCR buffer, 1.5 µl of $MgCl_2$ (50 mM), 1 µl of each primer, 2.5 U *Taq* DNA polymerase, 1 µl DNA template and an appropriate volume of nuclease-free water.

Run the samples in a thermal cycler as follows: 5 minutes at 94°C; 1 minute at 94°C, 1 minute at 45°C, 2.5 minutes at 72°C. (29 cycles); 10 minutes at 72°C then hold at 4°C until analysis.

Mix 10 µl of a sample with loading dye solution and load in 1% agarose gel in TBE (Tris/Borate/EDTA) buffer containing ethidium bromide or cyanine nucleic acid stain. Load a parallel lane with a 100 bp DNA-marker ladder. Separate the products at 100 V for 30–40 minutes and visualise using an UV transilluminator. Confirm the positive reactions according to the amplicon size.

A commercial PCR kit has been developed that allows detection of *Orthopoxvirus* DNA and contains a second 'conventional' amplification system, consisting of primers to the haemagglutinin (HA) gene of the orthopoxvirus. The amplicon can be sequenced and identified by comparison with already existing orthopoxvirus sequences.

1.6. Real-time PCR

A small quantity of test material (blood, skin lesion, tissue) is suspended in 200 µl of lysis buffer and 20 µl of proteinase K and incubated at 65°C for 1 hour. The lysed sample is extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25/24/1) and centrifuged at 8000 ***g*** for 10 minutes. The upper aqueous phase is transferred to a clean tube and the DNA is precipitated by adding 1/10 volume 3 M sodium acetate and 2.5 volumes of ice-cold absolute ethanol. The mixture is placed at –20°C for 1 hour followed by centrifugation at 13,000 ***g*** for 10 minutes. The supernatant is discarded and the pellet is washed with 0.5 ml 70% ethanol. The dried DNA pellet is resuspended in 30 µl of nuclease-free water. Alternatively, DNA can be extracted using commercially available DNA extraction kits.

For standardisation of the PCR, extracted DNA from the purified virus is used to amplify a 166-bp product from the HA gene of the camelpox virus genome (Pfeffer *et al.,* 1998b). PCR is performed in a 20-µl reaction containing 2 µl of 10× PCR reaction buffer, 1.6 µl of $MgCl_2$, (25 mM), 0.4 µl of dNTP (10 mM), (0.4 µl) of *Taq* polymerase, 5 units/µl, 2 µl DNA (approximately 50–100 ng), 6 pmol of each primer, and 2.5 pmol of each probe.

The cycling conditions are as follows:

Step	Temp.	Time	Acquisition mode	No. of cycles
Initial denaturation	95°C	10 minutes		1
Amplification	95°C	10 seconds		
	60°C	20 seconds single	Single	40
Melt	95°C	00[4]	Continuous	1
	60°C	30 seconds		
	95°C	00[3]		

A positive result is indicated by an amplification that gives a cycle threshold (Ct) value of 37 or less.

2. Serological tests

All the viruses in the genus *Orthopoxvirus* cross-react serologically. However, within the genus only camelpox virus can cause pox-like lesions in camels. Parapox and camelpox viruses do not cross-react and so infections of camelpox and camel orf can be distinguished serologically. Most of the conventional serological tests are very time- and labour-consuming, which makes them unsuitable for primary diagnosis. However, serological tests are a valuable tool for secondary confirmatory testing and retrospective epidemiological studies in those areas where vaccination against camelpox is not practised.

2.1. Virus neutralisation test

In this method the test sera are titrated against a constant titre of camelpox virus (100 $TCID_{50}$ [50% tissue culture infectious dose]) on Vero cells.

2.1.1. Test procedure

i) Sera are inactivated at 56°C for 30 minutes in a water bath.

ii) Sera are diluted in a twofold dilution series across the 96-well, flat-bottomed, cell-culture grade microtitre plate in serum-free cell culture medium (25 µl volumes). Individual serum controls, together with negative and known positive control sera, must also be included in each test.

iii) A dilution of stock virus made up to contain 100 $TCID_{50}$ per 25 µl is prepared using serum-free cell culture medium containing antibiotics.

iv) 25 µl of the appropriate stock virus dilution is added to every well containing 25 µl of each serum dilution, except the test serum control wells and cell control wells on each plate.

v) Plates are covered and incubated 1 hour at 37°C in 5% CO_2 atmosphere.

vi) A cell suspension is made from 3–4 days old Vero cells using a concentration that will ensure confluent monolayers in the microtitre plate wells within 18–24 hours after seeding.

vii) A volume of 100 µl of cell suspension is added to every well, the plates are sealed with tape and incubated at 37°C in 5% CO_2 atmosphere for 3–4 days.

viii) Plates are examined microscopically for CPE and results are recorded on a work sheet. Wells are scored as positive for neutralisation of virus if 100% of the cell monolayer is intact. The highest dilution of serum resulting in complete neutralisation of virus (no CPE) in half of the test wells is the 50% end-point titre of that serum. If necessary, the titre can be determined by the Spearman–Kärber method. A titre of 1/8 or greater is considered to be positive.

2.2. Enzyme-linked immunosorbent assay for the detection of antibodies against camelpox virus

The following procedure for the antibody ELISA for *Camelpox virus* is described by Azwai *et al*. (1996) and Pfeffer *et al*. (1998b). The following description gives general guidelines for the test procedure.

4 This is a continuous step, i.e. the temperature is increased to 95°C and then lowered to 60°C for 30 seconds and then again increased to 95°C before finishing the cycle.

2.2.1. Preparation of the antigen

i) Harvest the cell culture when 100% infected with camelpox virus. Freeze and thaw two to three times. Sonicate for 30 seconds at 80 Hz on ice to release the virus from the cells.

ii) Centrifuge at 1000 ***g*** for 10 minutes and collect the supernatant.

iii) Centrifuge the supernatant at 45,000 ***g*** at 4°C for 1 hour. Re-suspend the pellet in PBS.

iv) Add NaCl to a final concentration of 330 mM and polyethylene glycol (PEG 6000) to a final concentration of 7%.

v) Stir overnight at 4°C, centrifuge at 3000 ***g*** at 4°C for 10 minutes and wash the pellet twice with 15 mM NaCl.

vi) Freeze and thaw, and treat with 1% non-ionic detergent (Nonidet P40, Sigma) at 37°C for 3 hours.

vii) Freeze and thaw and centrifuge at 3000 ***g*** for 10 minutes at 4°C.

viii) Collect the supernatant and dialyse at least three times against PBS.

ix) Measure the protein concentration as described by Lowry *et al*. (1951).

x) Store the aliquots at –20°C.

2.2.2. Preparation of rabbit anti-camel IgG horseradish-peroxidase conjugate

Rabbit anti-llama and anti-camel IgG horseradish-peroxidase conjugates are commercially available. The method for producing monoclonal antibodies for camel IgM and IgG has been described by Azwai *et al*. (1995). However, rabbit anti-camel IgG horseradish-peroxidase can be replaced with a commercially available protein A-peroxidase, *Staphylococcus aureus*/horseradish conjugate.

i) Precipitate camel sera twice adding saturated ammonium sulphate to a final concentration of 40% (v/v) (29.6% ammonium sulphate [w/v]) at room temperature. Centrifuge at 12,000 ***g*** for 15 minutes and dissolve in PBS, pH 7.2. Dialyse against several changes of PBS overnight.

ii) Separate the immunoglobulins using gel filtration chromatography: a column (2.6 × 100 cm) can be used to separate the salt precipitated immunoglobulins (IgM and IgG) by size. Elution can be effected with PBS at 20 ml/hour and 6 ml fractions can be collected. Determine the protein concentrations by absorbance at 280 nm.

2.2.3. Antiserum production

Immunise rabbits with a subcutaneous injection of camel IgG emulsified in appropriate adjuvant. The animals should be immunised three times to booster antibody production. Collect the serum and store at –20°C until used.

2.2.4. Test procedure

i) Coat 96-well microtitre ELISA plates with prepared antigen at 1 µg/ml in carbonate/bicarbonate buffer, 0.05 M, pH 9.6 (100 µl per well).

ii) Incubate the ELISA plates in a humid chamber (100% humidity) at 37°C for 1 hour and then overnight at 4°C.

iii) Wash off the unbound antigen with PBS containing 0.05% Tween 20 (PBS/Tween) three times.

iv) Add 100 µl of test and control serum at a predetermined optimal dilution in blocking buffer (PBS containing 0.05% Tween 20, and 1% fat-free milk powder) in duplicate wells.

v) Incubate the plates for 30 minutes at 37°C.

vi) Wash the plates three times with PBS/Tween.

vii) Dilute rabbit anti-camel IgG horseradish-peroxidase conjugate or protein A-peroxidase, *Staphylococcus aureus*/horseradish conjugate at a predetermined working dilution in blocking buffer and add 100 µl into the wells.

viii) Incubate the plates at 37°C for 30 minutes.

ix) Wash the plates three times with PBS/Tween.

x) Make the reaction visible using 100 µl per well of chromogen 3.3' 5.5'-tetramethylbenzidine (TMB) with hydrogen peroxide and incubate 15 minutes at 37°C with shaking.

xi) Stop the reaction 10 minutes later by 2 M H_2SO_4 at a volume of 50 µl/well.

xii) Measure the values with a photometer at a wave length of 450 nm. Seropositivity can be calculated as values above the mean +2 standard deviations from negative control sera.

C. REQUIREMENTS FOR VACCINES

C1. Live attenuated vaccine

1. Background

1.1. Rationale and intended use of the product

Currently live attenuated and inactivated vaccines are commercially available. The live attenuated vaccine was prepared from a strain isolated from a dromedary camel calf that had generalised camelpox (Wernery, 2000). The live attenuated vaccine gives long-term protection against camelpox (Wernery & Zachariah, 1999). However, a booster vaccination is recommended for young animals at the age of 8–12 months, 2–3 months after the initial vaccination, to avoid interference by maternal antibodies. When inactivated vaccine is used, the animals must be vaccinated annually.

Guidelines for the production of the veterinary vaccines are given in Chapter 1.1.8 *Principles of veterinary vaccine production*.

2. Outline of production and minimum requirements for vaccines

2.1. Characteristics of the seed

2.1.1. Biological characteristics

Samples (such as crusty material from the nose, skin lesions or scabs) are collected from a camel calf showing generalised camelpox lesions. The samples are crushed in MEM with antibiotics, centrifuged, sterile-filtered and inoculated onto confluent Vero cells and on fetal camel skin cell line (Dubca). CPE is observed after 4 days of incubation at 37°C. When 80% of the cells are infected, cell culture is harvested, frozen and thawed. This procedure is done three times followed by sonication at 80 Hz on ice to release the virus. The suspension is clarified by centrifugation at 1000 ***g*** for 10 minutes. The supernatant is collected and the identity of camelpox virus is confirmed using different test methods. After the 10^{th} passage on Vero cells, a plaque test is carried out and the bigger plaque is chosen for the vaccine preparation. The plaque-purified virus is then passaged 110 times on Vero cells to attenuate the virus and designated as the master seed virus (MSV). The MSV is kept freeze dried and frozen at –80°C.

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

The purity and identity of the seed virus and the cells used for the vaccine production is proved. The seed virus is demonstrated free from contamination with adventitious viruses, bacteria, fungi or mycoplasma.

2.2. Method of manufacture

2.2.1. Procedure

The cells used for the production of vaccine preparation are prepared using a seed-lot system. The seed virus is cultured on Vero cells. When the cell monolayer is confluent, the cells are infected with the vaccine virus. Cell culture is harvested when the cells are 100% infected with camelpox virus. The supernatant is clarified, mixed with a stabiliser, bottled and freeze-dried.

2.2.2. Requirements for ingredients

Cell cultures and all animal origin products used in the production and maintenance of cells are proved free of adventitious viruses, bacteria, fungi and mycoplasma.

2.2.3. In-process controls

CPE is checked during cultivation of working virus seed. Uninoculated control cells should retain their morphology until the time of harvesting. Virus multiplication is demonstrated by titration with the harvested supernatant.

2.2.4. Final product batch tests

i) Sterility/purity

The procedure for testing for sterility and freedom from contamination of biological materials intended for veterinary use is described in chapter 1.1.9.

ii) Safety

Using the recommended route of administration, each batch of vaccine is tested in ten naive camels, using ten times the recommended dose per animal. The animals are observed for 7–14 days for any adverse reactions.

iii) Batch potency

The amount of virus present in the live attenuated vaccine is titrated on cell culture and the end titre is calculated.

2.3. Requirements for regulatory approval

2.3.1. Safety requirements

i) Target and non-target animal safety

Live attenuated camelpox vaccine causes no clinical signs in camelpox-susceptible Old Word camels and New World camels. Less than 1% of animals show a rise in temperature by a maximum of 1°C.

ii) Reversion-to-virulence

There are no reports of reversion to virulence by the live attenuated vaccine.

iii) Precautions

There is a low risk of human infection as camelpox is host specific. However, self-inoculation may lead to zoonotic camelpox virus infection with a vaccine virus.

2.3.2. Efficacy

Efficacy in susceptible animals is demonstrated in naive dromedaries. The experimental animals should be vaccinated twice with the live attenuated vaccine and 3 weeks after the last vaccination the camels should be challenged with a virulent camelpox field strain. The virulence of the challenge virus should be demonstrated by inoculation of the virus into unvaccinated control animals. The vaccinated animals should not show any clinical signs whereas the unvaccinated group should develop characteristic clinical signs of camelpox. The long-term immunity provided by the live attenuated vaccine can also be confirmed by challenging the vaccinated animals 6 years later. The efficacy of the live attenuated vaccine should be further evaluated by measuring the antibody levels against camelpox virus 21–30 days after vaccination using ELISA and virus neutralisation test.

The live attenuated vaccine can protect dromedaries from an infection for at least 6 years, but may be lifelong.

2.3.3. Stability

Vaccines should be stored at 4–8°C, with minimal exposure to light. The shelf life is determined by virus titration.

C2. Inactivated vaccine

1. Background

1.1. Rationale and intended use of the product

An inactivated vaccine has been available since 1992 and used mainly in North Africa to prevent the disease (El Harrak & Loutfi, 1999). The inactivated vaccine is prepared from a strain isolated during the 1984 outbreak in Morocco from a dromedary camel presenting with generalised camelpox (El Harrak *et al.,* 1991). The inactivated vaccine gives good protection against camelpox after a double injection administered at a 3- to 6-month interval followed by the annual booster. The vaccine is recommended from the age of 8–12 months to avoid interference by maternal antibodies.

Guidelines for the production of the veterinary vaccines are given in Chapter 1.1.8 *Principles of veterinary vaccine production*. The production of commercially available inactivated camelpox vaccine is described below.

2. Outline of production and minimum requirements for vaccines

2.1. Characteristics of the seed

2.1.1. Biological characteristics

Pox lesions from an adult dromedary camel presenting generalised camelpox infection are removed, crushed in PBS with antibiotics, centrifuged, sterile-filtered and inoculated onto the chorioallantoic membrane of specific pathogen free eggs for the first isolation (El Harrak *et al.,* 1991). The pox lesions obtained are passed on confluent Vero cells serially seven times. A CPE is routinely observed after 3–4 days of incubation at 35°C. When 80% of the cells are infected, viral suspension is harvested, frozen and thawed. This procedure is done twice to release the virus. The suspension is collected and the identity of the camelpox virus is proven using different test methods. The virus at its eighths passage level was designated the master seed virus (MSV) and named Laayoune T8 strain. The MSV is kept frozen at –80°C.

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

The purity, identity and titre of the seed virus as well as the purity of the Vero cells used for the vaccine production is proved. The seed virus is demonstrated free from contamination with adventitious viruses, bacteria, fungi or mycoplasma.

2.1.3. Validation as a vaccine strain)

Laayoune T8 strain was validated as a vaccine strain after three successive passages on Vero cells using the limiting dilution method to purify the virus. CPE characteristics and the virus titre are reproducible and freedom from extraneous agents proven.

2.2. Method of manufacture

2.2.1. Procedure

The seed virus is grown on Vero cells. Cells used for vaccine production are prepared using a seed-lot system. The infection with the seed virus takes place on confluent Vero cells in roller bottles or in bio-generators with micro-carriers. Viral suspension is harvested when the cells are 80% infected with camelpox virus. The virus is inactivated using betapropiolactone then mixed with aluminium hydroxide as an adjuvant and bottled.

2.2.2. Requirements for ingredients

The seed virus, cell cultures and all animal origin ingredients used in the production are proved free of adventitious viruses, bacteria, fungi and mycoplasma.

2.2.3. In-process controls

Cell sterility and purity are tested during the production process. The harvested virus is tested for sterility and infectious titre, the inactivated antigen is also checked for sterility and complete inactivation.

2.2.4. Final product batch tests

i) Sterility/purity

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

ii) Identity

Identity of the vaccine virus is confirmed by PCR on the inactivated final product.

iii) Safety

Using the recommended route of administration, each batch of vaccine is tested in two naive camels, using two times the recommended field dose per animal. The animals are observed for 14 days for any adverse reactions.

iv) Batch potency

The amount of virus present in the inactivated vaccine is measured by titration on cell culture before inactivation and by real time PCR carried out on antigen before addition of the adjuvant.

2.3. Requirements for regulatory approval

2.3.1. Manufacturing process

The inactivated camelpox vaccine is produced according to good manufacturing practices (GMP) standards and procedures recommended for inactivated veterinary vaccines.

2.3.2. Safety requirements

i) Target and non-target animal safety

Inactivated camelpox vaccine causes no clinical signs or rise in body temperature when administered to camelpox-susceptible animals. An inflammatory reaction due to the presence of the adjuvant may appear at the injection site with no influence on the animal's health.

ii) Reversion-to-virulence for attenuated/live vaccines and environmental considerations

Not applicable for inactivated vaccines.

iii) Precautions (hazards)

There are no precautions necessary as camelpox is host specific.

2.3.3. Efficacy requirements

Efficacy is demonstrated in six naive calf dromedaries challenged with a virulent field strain after two injections with the inactivated vaccine at a 3-month interval. The virulent strain is titrated on the animal skin comparatively on four vaccinated and two unvaccinated control animals. The obtained infectious titre on vaccinated animals is at least 1.5 log ID_{50} less than the titre obtained on control animals. In addition, vaccinated animals do not show any signs of generalised disease. The efficacy of the inactivated vaccine was also proven by the development of antibodies after vaccination that were evaluated by ELISA and the virus neutralisation test (El Harrak & Loutfi, 1999).

2.3.4. Vaccines permitting a DIVA strategy (detection of infection in vaccinated animals)

There is no vaccine available permitting a DIVA strategy for camelpox control.

2.3.5. Duration of immunity

The long-term immunity caused by the inactivated vaccine was also confirmed by challenging the vaccinated animals 1 year after the primary vaccination. Duration of the conferred immunity with inactivated vaccine lasts for a minimum of 1 year after double injection of the vaccine, as tested on young naïve dromedary camels. After multiple vaccinations, immunity may be longer in adult dromedaries; no vaccination failures reported in the field with animals not regularly vaccinated.

2.3.6. Stability

Vaccines should be stored at 4–8°C, with minimal exposure to light. The shelf life is 24 months.

REFERENCES

AZWAI S.M., CARTER S.D. & WOLDEHIWET Z. (1995). Monoclonal antibodies against camel (*Camelus dromedarius*) IgG, IgM and light chains. *Vet. Immunol. Immunopathol.*, **45**, 175–184.

AZWAI S.M., CARTER S.D., WOLDEHIWET Z. & WERNERY U. (1996). Serology of *Orthopoxvirus cameli* infection in dromedary camels: Analysis by ELISA and western blotting. *Comp. Immunol. Microbiol. Infect. Dis.*, **19**, 65–78.

BALAMURUGAN V., BHANUPRAKASH V., HOSAMANI M., JAYAPPA K.D., VENKATESAN G., CAUHAN B. & SINGH R.K. (2009). A polymerase chain reaction strategy for the diagnosis of camelpox. *J. Vet. Diagn. Invest.,* **21**, 231–237.

BERA B.C., SHANMUGASUNDARAM K., SANJAY BARUA, VENKATESAN G., NITIN VIRMANI, RIYESH T., GULATI B.R., BHANUPRAKASH V., VAID R.K., KAKKER N.K., MALIK P., MANISH BANSAL, GADVI S., SINGH R.V., YADAV V., SARDARILAL, NAGARAJAN G., BALAMURUGAN V., HOSAMANI M., PATHAK K.M.L. & SINGH R.K. (2011). Zoonotic cases of camelpox infection in India. *Vet. Microbiol*., **152**, 29–38.

BIEL ST.S. & GELDERBLOM H.R. (1999). Electron microscopy of viruses. Virus Culture – a Practical Approach, Cann, A.J. ed. Oxford University Press, UK, pp. 111–147.

COETZER J.A.W. (2004). Poxviridae. *In:* Infectious Diseases of Livestock, Second Edition, Vol. 2, Coetzer J.A.W. & Tustin R.C., eds. Oxford University Press Southern Africa, Cape Town, South Africa, 1265–1267.

DAVIES F.G., MUNGAI J.N. & SHAW T. (1975). Characteristics of Kenyan camelpox virus. *J. Hyg.*, **75**, 381–385.

EL HARRAK M. & LOUTFI C. (1999). Camel pox in the calf in Morocco. Identification of the virus. Vaccine Development and Application to Prophylaxis. Int. Workshop of Young Camel, Ouarzazate, Morocco, 24–26 October 1999. http://remvt.cirad.fr/CD/EMVT00_2.PDF

EL HARRAK M., LOUTFI C. & BERTIN F. (1991). Isolation and identification of camel poxvirus in Morocco. *Ann. Rech. Vet*., **22**, 95–98.

JOHANN S. & CZERNY C.-P. (1993). A rapid antigen capture ELISA for the detection of orthopox viruses. *J. Vet. Med.* [*B*], **40**, 569–581.

KINNE J., COOPER J.E. & WERNERY U. (1998). Pathological studies on camelpox lesions of the respiratory system in the United Arab Emirates (UAE). *J. Comp. Pathol.*, **118**, 257–266.

KRITZ B. (1982). A study of camelpox in Somalia. *J. Comp. Pathol.*, **92**, 1–8.

LOWRY O.H., ROSEBROUGH N.J., FARR A.L. & RANDALL R.J. (1951). Protein measurement with folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

MAYER A. & CZERNY C.-P. (1990). Chapter 4, Camelpox virus. *In:* Virus Infections of Vertebrates, Vol. 3, Virus Infections of Ruminants, Dinter Z. & Morein B., eds. Elsevier Science Publisher B.V., Amsterdam, The Netherlands, 19–22.

MEYER H., PFEFFER M. & RZIHA H.-J. (1994). Sequence alterations within and downstream of the A-type inclusion protein genes allow differentiation of *Orthopoxvirus* species by polymerase chain reaction. *J. Gen. Virol.*, **75**, 1975–1981.

MEYER H., ROPP S.L. & ESPOSITO J.J. (1997). Gene for A-type inclusion body protein is useful for a polymerase chain reaction assay to differentiate orthopoxviruses. *J. Virol. Methods*, **64**, 217–221.

NOTHELFER H.B., WERNERY U. & CZERNY C.P. (1995). Camel pox: antigen detection within skin lesions – Immunohistochemistry as a simple method of etiological diagnosis. *J. Camel Pract. Res.*, **2**, 119–121.

PFEFFER M., MEYER H., WERNERY U. & KAADEN O.-R. (1996). Comparison of camelpox viruses isolated in Dubai. *Vet. Microbiol.*, **49**, 135–146.

PFEFFER M., NEUBAUER H., WERNERY U., KAADEN O.-R. & MEYER H. (1998a). Fatal form of camelpox virus infection. *Vet. J.*, **155**, 107–109.

PFEFFER M., WERNERY U., KAADEN O.-R. & MEYER H. (1998b). Diagnostic procedures for poxvirus infections in camelids. *J. Camel Pract. Res.*, **5**, 189–195.

ROPP S.L., JIN Q., KNIGHT J.C. MASSUNG R.F. & ESPOSITO J.J. (1995). PCR strategy for identification and differentiation of smallpox and other orthopoxviruses. *J. Clin. Microbiol*., **33**, 2069–2076.

TANTAWI H.H., SABAN M.S., REDA I.M. & EL-DAHABY H (1974). Camelpox virus in Egypt I – Isolation and Characterization. *Bull. Epizoot. Dis. Africa*, **22**, 315–319.

WERNERY U. (2000). Production of an attenuated camelpox vaccine (Ducapox). *J. Camel Pract. Res*., **7**, 117–119.

WERNERY U., KINNE J. & SCHUSTER R.K. (EDS) (2014). Camelid Infectious Disorders, Third Edition. OIE, World Organisation for Animal Health, Paris, France, pp. 1–500.

WERNERY U., KAADEN O.-R. & ALI M. (1997a). Orthopox virus infections in dromedary camels in United Arab Emirates (U.A.E.) during winter season. *J. Camel Pract. Res.*, **4**, 51–55.

WERNERY U., MEYER H. & PFEFFER M. (1997b). Camel pox in the United Arab Emirates and its prevention. *J. Camel Pract. Res.*, **4**, 135–139.

WERNERY U. & ZACHARIAH R. (1999). Experimental camel pox infection in vaccinated and unvaccinated dromedaries. *J. Vet. Med.* [*B*] *Infect. Dis.*, **46**, 131–136.

YAGER J.A., SCOTT D.W. & WILCOCK B.P. (1991). Viral diseases of the skin. *In:* Pathology of Domestic Animals, Fourth Edition, Jubb K.V.F., Kennedy P.C. & Palmer N., eds. Academic Press, San Diego, California, USA, 629–644.

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NB: There is an OIE Reference Laboratory for camelpox (please consult the OIE Web site: https://www.oie.int/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact the OIE Reference Laboratories for any further information on diagnostic tests, reagents and vaccines for camelpox

NB: FIRST ADOPTED IN 2008. MOST RECENT UPDATES ADOPTED IN 2021.