CHAPTER 2.9.2.

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, causing economic impact through loss of production and sometimes death. The 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, respiratory and digestive tracts. 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. 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. Only one suspected case of human camelpox involving mild skin lesions has been described, underlining that camelpox is of no public health importance.

Identification 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 fastest method of laboratory confirmation of camelpox is by the demonstration of the characteristic, brick-shaped orthopox virions 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 on TEM as infection with both viruses has been reported to occur.

Camelpox can be confirmed by demonstration of the camelpox antigen in scabs and pox 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 polymerase chain reaction, 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 are available to identify camelpox. The tests used for the detection of the antibodies against camelpox virus include neutralisation, agar gel precipitation, haemagglutination, haemagglutination inhibition, complement fixation, fluorescent antibody and antibody-capturing ELISA.

Requirements for vaccines and diagnostic biologicals: 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. There are no standardised requirements for diagnostic biologicals.
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 (Bahrain, Iran, Iraq, Oman, Saudi Arabia, United Arab Emirates and Yemen), in Asia (Afghanistan and Pakistan), in Africa (Algeria, Egypt, Ethiopia, Kenya, Mauritania, Morocco, Niger, Somalia and Sudan) (9, 18) and in the southern parts of Russia and India. The disease is endemic in these countries and a pattern of sporadic outbreaks occurs with a rise in the seasonal incidence usually during the rainy season.

Camelpox is caused by Orthopoxvirus cameli virus which belongs to genus Orthopoxvirus within 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 nucleic acid is a double-stranded linear DNA. Virus replicates in the cytoplasm of the host cell, in so-called inclusion bodies. Camelpox virus haemagglutinates cockerel erythrocytes. However, the haemagglutination may be poor (4). Camelpox virus is ether resistant and chloroform sensitive (4, 15). The virus is sensitive to pH 3–5 and pH 8.5–10 (4). Poxviruses are susceptible to various disinfectants including 1% sodium hypochlorite, 1% sodium hydroxide, 1% peracetic acid, formaldehyde, 0.5–1% formalin and 0.5% quaternary ammonium compounds. The virus can be destroyed by autoclaving, boiling for 10 minutes and is killed by ultraviolet rays (245 nm wave length) in a few minutes (3).

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 (16). 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, respiratory and digestive tracts (7, 16).

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 due to secondary infections and septicaemia (16).

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 (20).

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, respiratory and digestive 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 affected areas by macrophages, necrosis and fibrosis (6, 13, 17).

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 (16). The incidence of disease is higher in males than females, and the mortality rate is greater in young animals than in adults (7). The mortality rate in adult animals is between 5% and 28% and in young animals between 25% and 100% (9).

Transmission is by either direct contact between infected and susceptible animals or indirect infection via a contaminated environment. The infection is usually achieved by inhalation or through skin abrasions. Virus is secreted in milk, saliva, and ocular and nasal discharges. Dried scabs shed from the pustules 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 (17). However, other potential vectors may be involved, such as biting flies and mosquitoes.

It has been suggested that different strains of camelpox virus may show some variation in their virulence (16). Restriction enzyme analysis of viral DNA allows isolates to be compared. However, no major differences from the vaccine strain have so far been demonstrated (17).
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 (16). Life-long immunity follows after natural infection. Live, attenuated vaccine provides protection against the disease for at least 6 years, probably longer (19). 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. Field reports of mild skin lesions in humans associated with camelpox have been made (3), but it appears that only one suspected case of human camelpox has been described (7), underlining that camelpox is of no public health importance.

B. DIAGNOSTIC TECHNIQUES

During the viremic 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 1–2 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. 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, 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. Identification of the agent

a) 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 best method for distinguishing clinical cases of camelpox and orf caused by camelpox and parapox viruses respectively, although the viruses can be differentiated by serological techniques and by PCR (9).

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 (12, 14).

○ 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 (12, 14).

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.
b) Virus isolation in cell cultures

Camelpox virus can be propagated in a large variety of cell cultures including the following cell lines: Vero, MA-104 and MS monkey kidney, and, baby hamster kidney (BHK) and the following primary cell cultures: lamb testis, lamb kidney, camel embryonic kidney, calf kidney, and chicken embryo fibroblast (4, 15).

The samples are prepared for virus isolation as described above in Section B.1.a.

- **Test procedure**

  Incubate 400 µl of the supernatant for 1 hour at room temperature and overnight at 4°C. Filter the supernatant through a 0.45 µm filter and inoculate into a 25 cm² 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 10 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 10–12 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 (15). The growth of camelpox virus on a cell culture can be confirmed by TEM, PCR or antigen-capture enzyme-linked immunosorbent assay (ELISA) (5).

c) Virus isolation on chorioallantoic membrane of embryonated chicken eggs

Camelpox virus can be isolated on the chorioallantoic membrane (CAM) of 11–13-day old embryonating chicken eggs. The eggs should be incubated at 37°C degrees 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 degrees. If the eggs are incubated at 34.5°C the pocks are flatter and a haemorrhagic centre may develop (15).

d) Immunohistochemistry

Immunohistochemistry for the detection of the infectious agent of camelpox is a relatively fast method and can be used instead of electron microscopy for establish a tentative diagnosis (11). Almost any polyclonal antibody against vaccinia virus is likely to produce reasonable results in this test because of the wide homology between vaccinia and camelpox viruses (11).

- **Test procedure**

  The following procedure for immunohistochemistry is described by Kinne et al. (6) and Pfeffer et al. (14). The entire skin pustule should be collected for the immunohistochernical 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 the glass slides. Treat the deparaffinised and dehydrated sections with 3% H₂O₂, 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, 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 (ABC-kit, Dako, Glostrup, Denmark). Wash with PBS for 5 minutes and incubate with streptavidin-peroxidase for 30 minutes. Wash again with PBS for 5 minutes and add diaminobenzidine as chromogen for 10 minutes.

e) Polymerase chain reaction

The PCR is a fast and sensitive method for the detection of orthopoxviral DNA. A generic PCR assay, described by Meyer et al. (10), 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.

- **Test procedure**

  Suspend a small aliquot of crusted scabs in 90 µl of lysis solution (50 mM Tris/HCl, pH 8.0, 100 mM Na₂EDTA, 100 mM NaCl, 1% sodium dodecyl sulphate) and add 10 µl of proteinase K (20 mg/ml, Invitrogen). 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/isoamylalcohol (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/isoamylalcohol (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.

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.

Incubate the samples in a thermal cycler: first cycle: 5 minutes at 94°C (initial denaturation step), second cycle: 1 minute at 94°C, 1 minute at 45°C, 2.5 minutes at 72°C. Repeat the second cycle 29 times. Last cycle: 10 minutes at 72°C (final elongation step) and 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. 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 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.

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 not suitable 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.

a) Serum 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]).

- **Test procedure**
  - i) Mark the microtitre plates.
  - ii) Dilute the test sera, positive and negative serum controls 1/5 in Dulbecco’s Modified Eagle’s Medium (DMEM).
  - iii) Inactivate the sera at 56°C for 30 minutes.
  - iv) Add the growth medium (DMEM containing 5–10% of fetal calf serum and antibiotics) into the wells:
    - 100 µl into rows A to H, columns 2 to 6 (test serum, positive and negative serum controls) and rows A to D, columns 7 to 12 (virus control rows),
    - 200 µl into rows G to H, columns 7 to 12 (cell control rows).
  - v) Add the diluted and inactivated test sera, positive and negative control sera: 200 µl/well into rows A to H, column 1 (duplicate rows for each sample).
  - vi) Collect 100 µl from the test serum wells (1/5 dilution) and prepare twofold dilutions (1/10, 1/20, 1/40, 1/80, 1/160) of the serum samples on the microtitre plate using a multichannel pipette. Discard 100 µl from the last wells.
  - vii) Prepare 100 TCID\(_{50}\)/100 µl dilution of the virus suspension with a known titre of over \( \log_{10} 6 \) TCID\(_{50}\) to be used as a working virus seed.
  - viii) Prepare tenfold dilution series from working virus seed (10\(^{-1}\), 10\(^{-2}\), 10\(^{-3}\), 10\(^{-4}\)).
  - ix) Add 100 µl of each virus dilution into the virus control rows:
    - Working virus seed in rows A to D, columns 7 and 8,
    - 10\(^{-1}\) dilution of the working virus seed into rows A to D, column 9,
    - 10\(^{-2}\) dilution of the working virus seed into rows A to D, column 10,
    - 10\(^{-3}\) dilution of the working virus seed into rows A to D, column 11,
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- $10^{-4}$ dilution of the working virus seed into rows A to D, column 12.

x) Add 100 µl of working virus seed into each well in test serum, positive and negative serum control rows.

xi) Incubate the microtitre plate at 37°C with 5% CO$_2$ for 1 hour.

xii) Add 80 µl of suitable cell suspension, such as lamb testis cells, at a concentration of 480,000 cells/ml into each well.

xiii) Incubate at 37°C with 5% CO$_2$.

xiv) Read the results using an inverted microscope. Examine the microtitre plates daily. Serum antibody titres are calculated and recorded when CPE is evident in the virus control wells. The test is valid if the virus titre is approximately 100 TCID$_{50}$. The antibody titre is determined by the Spearman–Kärber method.

b) Enzyme-linked immunosorbent assay for the detection of antibodies against camelpox virus

The following procedure for antibody-detecting ELISA test for Orthopoxvirus camelid is described by Azwai et al. (2) and Pfeffer et al. (14). The following description gives general guidelines for the test procedure.

- Preparation of the antigen

  i) Harvest the cell culture when 100% infected with camelpox virus. Freeze and thaw two to three times.

  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 (8).

  x) Store the aliquots at -20°C.

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

Unfortunately rabbit anti-camel IgG horseradish-peroxidase conjugate is not commercially available. The method for producing monoclonal antibodies for camel IgM and IgG has been described by Azwai et al. (1). However, rabbit anti-camel IgG horseradish-peroxidase can be replaced with commercially available product in which the antibodies are raised against IgG of the New World camelids and conjugated with fluorescein (Fluorescein Conjugated Camelid IgG, Kent Laboratories, Triple J Farms, USA).

  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: an ACA-34 (LBK) 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.

- 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.

- Test procedure

Non-activated, 96-well, microtitre ELISA plates, such as Immulon 2 supplied by Dynatech can be used.
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C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

A live attenuated vaccine, Ducapox, is manufactured by Highveld Biologicals, Onderstepoort, South Africa and an inactivated vaccine by Biopharma, Rabat, Morocco. A live attenuated vaccine gives long-term protection against camelpox (19). However, a booster vaccination is recommended for young animals vaccinated before the age of 6–9 months. 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.

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