

## PESTE DES PETITS RUMINANTS

---

### SUMMARY

*Peste des petits ruminants (PPR), is an acute contagious disease caused by a Morbillivirus in the family Paramyxoviridae. It affects mainly sheep and goats and occasionally small ruminants living in the wild. PPR occurs in Africa in countries lying between the Equator and the Sahara, in the Arabian Peninsula, throughout most of the Near East and Middle East, and in south-west Asia.*

*The clinical disease resembles rinderpest in cattle. It is usually acute and characterised by serous ocular and nasal discharges. PPR is characterised by severe pyrexia, erosive lesions on different mucous membranes and particularly in the mouth, diarrhoea and pneumonia. At necropsy, characteristic zebra markings may occur in the large intestine, but are not a consistent finding. Lesions also occur in the lungs showing congestion or bronchopneumonia when associated with bacterial infection.*

*The disease must be differentiated from rinderpest, bluetongue, foot and mouth disease and other exanthemous conditions.*

**Identification of the agent:** *The collection of specimens at the correct time is important to achieve diagnosis by virus isolation and they should be obtained in the acute phase of the disease when clinical signs are still apparent. The specimens can be swabs of conjunctival discharges, nasal secretions, buccal and rectal mucosae, and unclotted blood.*

*Rapid diagnosis is done by immunocapture enzyme-linked immunosorbent assay (ELISA), counter immunoelectrophoresis and agar gel immunodiffusion. Polymerase chain reaction may also be used.*

**Serological tests:** *The serological tests that are routinely used are the virus neutralisation and the competitive ELISA.*

**Requirements for vaccines and diagnostic biologicals:** *In the past, control of PPR was ensured through vaccination with the rinderpest tissue culture vaccine because of the existence of a strong antigenic relationship between PPR and rinderpest viruses. The use of this heterologous vaccine has been abandoned in favour of the live attenuated PPR virus vaccine, which is now widely commercially available.*

### A. INTRODUCTION

Peste des petits ruminants (PPR) is an acute viral disease of small ruminants characterised by fever, oculonasal discharges, stomatitis, diarrhoea and pneumonia with foul offensive breath. Infected animals present clinical signs similar to those of rinderpest in cattle, from which it must be differentiated. Because of the respiratory signs, PPR can be confused with contagious caprine pleuropneumonia (CCPP) or pasteurellosis. In many cases, pasteurellosis is a secondary infection of PPR, a consequence of the immunodepression that is induced by the causal agent of PPR, the PPR virus (PPRV). PPRV is transmitted mainly by aerosols between animals living in close contact (20).

On the basis of its similarities to the viruses of rinderpest, canine distemper and measles, the PPRV has been classified within the genus Morbillivirus in the family Paramyxoviridae (16). Virus members of this group have six structural proteins: the nucleocapsid protein (Np), which encapsulates the virus genomic RNA, the phosphoprotein (P), which associates with the polymerase (L for large protein) protein, the matrix (M) protein, the fusion (F) protein and the haemagglutinin (H) protein. The matrix protein, intimately associated with the internal face of the viral envelope, makes a link between the nucleocapsid and the virus external glycoproteins: H and F, which are responsible, respectively, for the attachment and the penetration of the virus into the cell to be infected. PPR was first described in Côte d'Ivoire (14), but it occurs in most African countries south of the Sahara and north of the

equator (20), and in nearly all Middle Eastern countries up to Turkey (13, 21, 26, 27, 37). PPR is also wide-spread in India and south-west Asia (34).

The natural disease affects mainly goats and sheep, but it is usually more severe in goats where it causes heavy losses and is only occasionally severe in sheep. It is generally admitted that cattle can only be infected subclinically. However, in poor conditions it might be possible that cattle develop lesions following PPRV infection, clinical signs of which would be ascribed to rinderpest. Indeed, in the 1950s, disease and death were recorded in calves experimentally infected with PPRV-infected tissue (25). Moreover, PPRV was isolated from an outbreak of rinderpest-like disease in buffaloes in India in 1995 (17). It was also suspected to be involved in the epizootic disease that affected one-humped camels in Ethiopia in 1995–1996 (28, 29). Indeed PPRV antigen and PPRV nucleic acid were detected in some pathological samples collected during that outbreak, but no live virus was isolated. Cases of clinical disease have been reported in wildlife resulting in deaths of gazelles in captivity (11, 13). The American white-tailed deer (*Odocoileus virginianus*) can be infected experimentally (18).

The incubation period is 4–6 days, but may range between 3 and 10 days. The clinical disease is acute, with a pyrexia up to 41°C that can last for 3–5 days; the animals become depressed, anorexic and develop a dry muzzle. The serous oculonasal discharges become progressively mucopurulent and, if death does not ensue, persist for around 14 days. Within 4 days of the onset of fever, the gums become hyperaemic, and erosive lesions develop in the oral cavity with excessive salivation. These lesions may become necrotic. A watery blood-stained diarrhoea is common in the later stage. Pneumonia, coughing, pleural rales and abdominal breathing also occur. The morbidity rate can be up to 100% with a mortality rate of up to 100% in severe cases. However, this may not exceed 50% during milder outbreaks. A tentative diagnosis of PPR is made on these clinical signs, but laboratory confirmation is required for differential diagnosis with other diseases with similar signs.

At necropsy, the lesions are very similar to those observed in cattle affected with rinderpest, except prominent crusty scabs along the outer lips and severe interstitial pneumonia frequently occur with PPR. Erosive lesions may extend from the mouth to the reticulo–rumen junction. Characteristic linear haemorrhages or zebra stripes occur in the large intestine, commonly at the caeco–colic junction, but they are not a consistent finding; necrotic or haemorrhagic enteritis is usually present. Lymph nodes are enlarged, the spleen may show necrotic lesions, and there is an apical pneumonia.

There are no known health risks to humans working with PPRV as no report of human infection with the virus exists.

## **B. DIAGNOSTIC TECHNIQUES**

### **1. Identification of the agent**

#### **a) Collection of samples**

In live animals, swabs are made of the conjunctival discharges and from the nasal and buccal mucosae. During the very early stage of the disease, whole blood is also collected in anticoagulant for virus isolation, polymerase chain reaction (PCR) and haematology. At necropsy (two to three animals), lymph nodes, especially the mesenteric and bronchial nodes, lungs, spleen and intestinal mucosae should also be collected aseptically, chilled on ice and transported under refrigeration. Fragments of organs collected for histopathology are placed in 10% formalin. At the end of the outbreak, blood can be collected for serological diagnosis.

#### **b) Agar gel immunodiffusion**

Agar gel immunodiffusion (AGID) is a very simple and inexpensive test that can be performed in any laboratory and even in the field. Standard PPR viral antigen is prepared from mesenteric or bronchial lymph nodes, spleen or lung material and ground up as 1/3 suspensions in buffered saline. These are centrifuged at 500 **g** for 10–20 minutes, and the supernatant fluids are stored in aliquots at –20°C. The cotton material from the cotton bud used to collect eye or nasal swabs is removed using a scalpel and inserted into a 1 ml syringe. With 0.2 ml of phosphate buffered saline (PBS), the sample is extracted by repeatedly expelling and filling the 0.2 ml of PBS into an Eppendorf tube using the syringe plunger. The resulting eye/nasal swab extracted sample, like the tissue ground material prepared above, may be stored at –20°C until used. They may be retained for 1–3 years. Negative control antigen is prepared similarly from normal tissues. Standard antiserum is made by hyperimmunising sheep with 1 ml of PPRV with a titre of 10<sup>4</sup> TCID<sub>50</sub> (50% tissue culture infective dose) per ml given at weekly intervals for 4 weeks. The animals are bled 5–7 days after the last injection (9). Standard rinderpest rabbit hyperimmune antiserum is also effective in detecting PPR antigen.

- i) Dispense 1% agar in normal saline, containing thiomersal (0.4 g/litre) or sodium azide (1.25 g/litre) as a bacteriostatic agent, into Petri dishes (6 ml/5 cm dish).
- ii) Wells are punched in the agar following a hexagonal pattern with a central well. The wells are 5 mm in diameter and 5 mm apart.
- iii) The central well is filled with positive antiserum, three peripheral wells with positive antigen, and one well with negative antigen. The two remaining peripheral wells are filled with test antigen, such that the test and negative control antigens alternate with the positive control antigens.
- iv) Usually, 1–3 precipitin lines will develop between the serum and antigens within 18–24 hours (10). These are intensified by washing the agar with 5% glacial acetic acid for 5 minutes (this procedure should be carried out with all apparently negative tests before recording a negative result). Positive reactions show lines of identity with the positive control antigen.

Results are obtained in one day, but the test is not sensitive enough to detect mild forms of PPR due to the low quantity of viral antigen that is excreted.

**c) Counter immunoelectrophoresis**

Counter immunoelectrophoresis (CIEP) is the most rapid test for viral antigen detection (24). It is carried out on a horizontal surface using a suitable electrophoresis bath, which consists of two compartments connected through a bridge. The apparatus is connected to a high-voltage source. Agar or agarose (1–2%, [w/v]) dissolved in 0.025 M barbitone acetate buffer is dispensed on to microscope slides in 3-ml volumes. From six to nine pairs of wells are punched in the solidified agar. The reagents are the same as those used for the AGID test. The electrophoresis bath is filled with 0.1 M barbitone acetate buffer. The pairs of wells in the agar are filled with the reactants: sera in the anodal wells and antigen in the cathodal wells. The slide is placed on the connecting bridge and the ends are connected to the buffer in the troughs by wetted porous paper. The apparatus is covered, and a current of 10–12 milliamps per slide is applied for 30–60 minutes. The current is switched off and the slides are viewed by intense light: the presence of 1–3 precipitation lines between pairs of wells is a positive reaction. There should be no reactions between wells containing the negative controls.

**d) Immunocapture enzyme-linked immunosorbent assay**

The immunocapture enzyme-linked immunosorbent assay (ELISA) (22) using three monoclonal antibodies (MAb) anti-N protein, allows a rapid differential identification of PPR or rinderpest viruses, and this is of great importance as the two diseases had until recently a similar geographical distribution and may affect the same animal species.

- i) Microtitre ELISA plates (e.g. high adsorption capacity Nunc Maxisorb) are coated with 100 µl of a capture MAb solution (diluted according to the instructions of the Reference Laboratory providing the kit). This MAb reacts with both rinderpest virus and PPRV.
- ii) After washing, 50 µl of the sample suspension is added to four wells, and control wells are filled with buffer.
- iii) Immediately, 25 µl of a detection biotinylated MAb for PPR and 25 µl of streptavidin/peroxidase are added to two wells, and 25 µl of a detection MAb for rinderpest and 25 µl of streptavidin/peroxidase are added to the two other wells.
- iv) The plates are incubated at 37°C for 1 hour with constant agitation.
- v) After three vigorous washes, 100 µl of ortho-phenylenediamine (OPD) in hydrogen peroxide is added, and the plates are incubated for 10 more minutes at room temperature.
- vi) The reaction is stopped by the addition of 100 µl of 1 N sulphuric acid, and the absorbance is measured at 492 nm on a spectrophotometer/ELISA reader.

The cut-off above which samples are considered to be positive is calculated from each blank (PPR blank and rinderpest blank) as three times the mean absorbance values.

A sandwich ELISA can also be performed: the sample is first allowed to react with the detection MAb and the immunocomplex is then captured by the second MAb adsorbed on to the ELISA plate.

The test is very specific and sensitive (it can detect  $10^{0.6}$  TCID<sub>50</sub>/well for the PPRV and  $10^{2.2}$  TCID<sub>50</sub> for the rinderpest virus). The results are obtained in 2 hours.

Another immunocapture test, based on the use of a single MAb anti-H, has been described (31).

**e) Nucleic acid recognition methods**

cDNA <sup>32</sup>P labelled clones have been used to differentiate PPR and rinderpest (6), but their use in routine diagnosis is not recommended due to the short half-life of the <sup>32</sup>P and the need for special equipment to protect the users.

A reverse transcription PCR (RT-PCR) technique based on the amplification of the Np and F protein genes has been developed for the specific diagnosis of PPR (5, 12, 33). This technique is very sensitive compared with other tests and results are obtained in 5 hours, including the RNA extraction. The OIE and FAO<sup>1</sup> Reference Laboratory for PPR in France (see Table given in Part 3 of this *Terrestrial Manual*) can advise on the use of this technique. A multiplex RT-PCR, based on the amplification of fragments of N and M protein genes, has been reported (15). Another format of the N gene-based RT-PCR has also been described (19). Instead of analysing the amplified product – the amplicon – by agarose gel electrophoresis, it is detected on a plate by ELISA through the use of a labelled probe. This new format, RT-PCR-ELISA, is ten times for sensitive than the classical RT-PCR.

**f) Culture and isolation methods**

Even when diagnosis has been carried out by rapid techniques, the virus should always be isolated from field samples in tissue cultures for further studies (10, 20).

PPRV may be isolated in primary lamb kidney or in African green monkey kidney (Vero) cell tissue cultures. Monolayer cultures are inoculated with suspect material (swab material, buffy coat or 10% tissue suspensions) and examined daily for evidence of cytopathic effect (CPE). The CPE produced by PPRV can develop within 5 days and consists of cell rounding and aggregation culminating in syncytia formation in lamb kidney cells. In Vero cells, it is sometimes difficult to see the syncytia. If they exist, they are very small. However, in stained, infected Vero cells, small syncytia are always seen. Syncytia are recognised by a circular arrangement of nuclei giving a 'clock face' appearance. Cover-slip cultures may give a CPE earlier than day 5. There are also intracytoplasmic and intranuclear inclusions. Some cells are vacuolated. Similar cellular changes may be seen in stained histopathological sections of infected tissues. After 5–6 days, blind passages should always be carried out as CPE may take time to appear.

**g) Other virus detection techniques**

Other virus detection techniques have potential benefits, but they are not yet widely used. While virus isolation needs pathological samples to be kept in cold conditions until the start of their processing, it is possible to keep them at ambient temperature in a formalin-fixed solution and later analyse them directly by immunofluorescence (IF) or immunochemical test (3, 4, 35). IF has been used successfully on conjunctival smears and tissues collected at necropsy; the smears are fixed in cold acetone. It has now been demonstrated that unlike the rinderpest virus but like the measles virus, PPRV has haemagglutination capability. This characteristic has been used for specific, rapid and inexpensive diagnosis of PPR infection (17, 39).

**2. Serological tests**

Goats and sheep infected with PPRV develop antibodies that may be demonstrated to support a diagnosis by the antigen-detection tests. Tests that are routinely used are the virus neutralisation (VN) test and the competitive ELISA.

**a) Virus neutralisation (the prescribed test for international trade)**

This test is sensitive and specific, but it is time-consuming. The standard neutralisation test is carried out in roller-tube cultures of primary lamb kidney cells, or Vero cells when primary cells are not available.

- i) Dilute 1 ml of inactivated serum in a twofold dilution series and mix with a stock virus suspension containing approximately 10<sup>3</sup> TCID<sub>50</sub>/ml.
- ii) Incubate the virus/serum mixtures either for 1 hour at 37°C or overnight at 4°C.
- iii) Inoculate 0.2 ml of the mixture into each of five roller tubes, followed immediately by 1 ml of Vero cell suspension in growth medium at a rate of 2 × 10<sup>5</sup> cells/ml.
- iv) Incubate the sloped tubes for 3 days at 37°C.
- v) Discard the tubes showing virus-specific CPE; replace the medium in the remaining tubes with maintenance medium, and roll the tubes for a further 7 days. The virus-challenge dose is acceptable if

---

1 Food and Agriculture Organization of the United Nations.

it falls between  $10^{1.8}$  and  $10^{2.8}$  TCID<sub>50</sub>/tube. Any detectable antibody at a dilution of 1/8 is considered to be positive.

Usually, a cross-neutralisation test is carried out with rinderpest virus and a serum is considered to be positive for PPR when the neutralisation titre is at least twofold higher for PPR than for rinderpest.

Instead of using the roller tubes, the VN test can also be performed in 96-well microtitre plates (30).

**b) Competitive enzyme-linked immunosorbent assay**

Competitive ELISA based on the use of MAb anti-nucleoprotein and a recombinant nucleoprotein produced in the baculovirus has been described (23).

- i) Coat microtitre plates (e.g. high adsorption capacity Nunc Maxisorb) with 50 µl of a predetermined dilution of N-PPR protein (produced by a recombinant baculovirus) for 1 hour at 37°C with constant agitation.
- ii) Wash the plates three times and blot dry.
- iii) Distribute 45 µl of blocking buffer (PBS + 0.5% Tween 20 + 0.5 fetal calf serum) to all wells, and then add 5 µl of test sera to test wells (at a final dilution of 1/20) and 5 µl of the different control sera (strong positive, weak positive and negative serum) to control wells.
- iv) Add 50 µl of MAb diluted 1/100 in blocking buffer, and incubate at 37°C for 1 hour.
- v) Wash the plates three times and blot dry.
- vi) Add 50 µl of anti-mouse conjugate diluted 1/1000, and incubate at 37°C for 1 hour.
- vii) Wash the plates three times.
- viii) Prepare OPD in hydrogen peroxide solution. Add 50 µl of substrate/conjugate mixture to each well. Stop the reaction after 10 minutes with 50 µl of 1 M sulphuric acid.
- ix) Read on an ELISA reader at 492 nm.

The absorbance is converted to percentage inhibition (PI) using the formula:

$$PI = 100 - (\text{absorbance of the test wells} / \text{absorbance of the MAb control wells}) \times 100$$

Sera showing PI greater than 50% are positive.

Two other competitive ELISA techniques, based on the use of monoclonal anti-haemagglutinin (H), have also been described (1, 32).

## **C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS**

Sheep and goats that recover from PPR develop an active immunity against the disease. As antibodies have been demonstrated 4 years after infection, this immunity is probably life-long (9). A homologous PPR vaccine is available. In 1998, the OIE International Committee endorsed the use of this vaccine in countries that have decided to follow the 'OIE pathway' for epidemiological surveillance for rinderpest in order to avoid confusion when serological surveys are performed. There have been two published reports on the preliminary results of the development of recombinant capripox-based PPR vaccine able to protect against both capripox and PPR (2, 7). The production of the commercially available attenuated PPRV vaccine is described here.

### **1. Seed management**

#### **a) Characteristics of the seed**

The PPRV vaccine Nigeria 75/1 strain is a live vaccine cultured in Vero cells. The original strain of the virus was isolated in Nigeria in 1975 (36). It has been attenuated by serial passages in Vero cell cultures (8). The strain provided for vaccine production is the 70th passage in Vero cells (PPRV 75/1 I.K6 BK2 Vero 70). It is stored in freeze-dried form at -20°C and may be obtained from Reference Laboratories (see Table given in Part 3 of this *Terrestrial Manual*). Tests of vaccine activity show that it retained the ability to protect (at a dose of  $10^3$  TCID<sub>50</sub>) up to the 120th passage in Vero cells, the latest passage tested so far.

#### **b) Method of culture**

##### **o Cells**

PPR vaccine is produced in Vero cells, which must be free from all bacterial, fungal and viral contamination.

o **Culture medium**

The culture medium consists of minimal essential medium (MEM) supplemented with antibiotics (for example penicillin + streptomycin at final concentrations of 100 IU [International Units]/ml and 100 µg/ml, respectively), and an antifungal agent (nystatin [Mycostatin] at a final concentration of 50 µg/ml). The medium is enriched with 10% fetal calf serum (complete medium) for cell growth. This proportion of serum is reduced to 2% for maintenance medium when the cell monolayer is complete.

o **Primary seed batch of vaccine virus**

This consists of virus in its 70th passage in Vero cells (PPRV 75/1 LK6 BK2 Vero 70). The freeze-dried contents of a flask from the seed bank are reconstituted with 2 ml of sterile water (or cell culture medium without serum). This liquid is mixed with Vero cells suspended in complete culture medium to provide at least 0.001 TCID<sub>50</sub> per cell. Cell culture dishes are filled with this virus/cell mixture (around  $2 \times 10^7$  Vero cells in a 175 cm<sup>2</sup> dish), and are incubated at 37°C. The cultured cells are examined regularly to detect a CPE. The medium is renewed every 2 days, reducing the proportion of serum to 2% once the cell monolayer is complete. Virus is first harvested when there is 40–50% CPE. This viral suspension is stored at –70°C. Successive harvesting is made every 2 days until the CPE reaches 70–80%, which is the time for final freezing of the culture dishes (in general, at least two further harvestings can be made before final freezing of the culture dishes). All suspensions of virus collected are submitted to two freeze–thaw cycles, then added to form a single batch, which serves as the primary seed batch. This is divided into small volumes in bottles and stored at –70°C. The contents of five flasks are thawed and titrated (minimum titre required: 10<sup>5</sup> TCID<sub>50</sub>/ml). It is best to freeze-dry this seed in order to store it at –20°C. In this case it will be necessary to titrate the freeze-dried virus (five bottles). A batch made up in this way must pass all tests for sterility.

When preparing seed batches, it is important to avoid infecting the cells with high doses of virus (high multiplicity of infection), as this will lead to accumulation of defective particles in the viral suspension produced, which will diminish the titre of subsequent products. On the other hand, very weak multiplicity of infection (e.g. 0.0001) will prolong the culture time.

o **Preparation of the working seed batch**

This is done under the same conditions as for the primary seed batch. A large stock of virus is formed, from which the final vaccine will be produced. This batch is distributed into receptacles and stored at –70°C. It must satisfy tests for sterility. Five samples are titrated (minimum titre required: 10<sup>6</sup> TCID<sub>50</sub>/ml).

c) **Validation as a vaccine**

It is necessary to confirm or rule out the presence of PPRV in the product under test. For this purpose, anti-PPR serum is used to neutralise the virus in cell culture.

o **Test procedure**

- i) Mix the contents of two vaccine bottles with sterile double-distilled water to provide a volume equal to the volume before freeze-drying.
- ii) Make tenfold dilutions of the reconstituted vaccine in serum-free culture medium (0.5 ml of viral suspension + 4.5 ml of medium).
- iii) Make two series of mixtures for virus dilutions from each bottle on a 96-well plate as follows:

Series 1:	Dilutions of viral suspension:	–1	–2	–3	–4
	Viral suspension (in µl)	50	50	50	50
	Culture medium (in µl)	50	50	50	50
Series 2:	Dilutions of viral suspension:	–1	–2	–3	–4
	Viral suspension (in µl)	50	50	50	50
	PPR antiserum (in µl)	50	50	50	50

(Note: PPR antiserum used for this purpose is prepared in goats and freeze-dried. It is reconstituted with 1 ml of sterile double-distilled water in a dilution of 1/10.)

- iv) Incubate the mixtures at 37°C for 1 hour
- v) Add to each well 100 µl of cells suspended in complete culture medium (30,000 cells/well).
- vi) Incubate the microplate at 37°C in the presence of CO<sub>2</sub>.
- vii) Read the plate after 10–15 hours of incubation.

Normally a CPE is present only in the wells containing cells infected with the mixture of virus and culture medium. If it is detected in the wells of Series 2, it will be necessary to identify PPRV by immunofluorescence, using a PPR MAb, or by immunocapture (specific PPR MAb, and the immunocapture test kit are available from the OIE Reference Laboratory for PPR in France [see Table given in Part 3 of this

*Terrestrial Manual*). If this identification confirms the presence of PPRV, the PPR antiserum used must have been too weak, or the batch must be changed. If immunofluorescence or immunocapture is negative, a viral contaminant must be present, and the material under test must be destroyed.

## **2. Method of manufacture**

### **a) Vaccine production**

This operation is performed on a larger scale. Cells can be infected with virus at a multiplicity of infection as before or with high doses, e.g. up to 0.01. Products of the various harvests, after two freeze–thaw cycles, are brought together (to form the final product) and stored at –70°C pending the results of titration and tests for sterility. If these results are satisfactory, the vaccine is freeze-dried.

### **b) Freeze-drying**

The freeze-drying medium (Weybridge medium) is composed of 2.5% (w/v) lactalbumin, 5% (w/v) sucrose and 1% (w/v) sodium glutamate, pH 7.2.

This medium is added to an equal volume of viral suspension for freeze-drying (which may have been diluted beforehand to provide the desired number of vaccine doses per bottle). The resulting mixture is kept cool, homogenised, then distributed into bottles and freeze-dried. At the end of a freeze-drying cycle, the probe is adjusted and kept at 35°C for 4 hours. Once this operation has been completed, the bottles are capped under vacuum. Randomly selected samples (e.g. 5%) of this final batch are submitted to tests for innocuousness, efficacy and sterility, and residual moisture is estimated by the Karl Fisher method (optimum  $\leq 3.5\%$ ). If the tests give unsatisfactory results, the entire batch is destroyed.

## **3. In-process control**

Cells used in cultures must be checked for normal appearance and shown to be free from contaminating viruses, especially bovine viral diarrhoea virus. A virus titration must be undertaken on the seed lot: using MEM (serum-free) medium, a series of tenfold dilutions is made (0.5 ml virus + 4.5 ml diluent) down to  $10^{-6}$  of the product to be titrated. Vero cells from one flask are trypsinised and suspended in complete culture medium at 300,000/ml. They are distributed on a 96-well plate (30,000 cells per well, equivalent to 100  $\mu$ l of cell suspension). Then, 100  $\mu$ l of virus diluted tenfold is added to the cells (dilutions ranging from  $10^{-2}$  to  $10^{-6}$ ). One row of wells serves as a control for uninfected cells to which virus-free culture medium (100  $\mu$ l) is added. The plate is incubated at 37°C in the presence of CO<sub>2</sub>. The plates are read (by examining for CPE) 10–15 days after infection.

Virus titre is determined by the Spearman–Kärber method. The minimum titre per dose is  $10^{2.5}$ .

## **4. Batch control**

### **a) Identity**

The contents of one container from each filling lot must be checked for identity by culture after neutralisation with specific antiserum.

### **b) Sterility**

This consists of testing for viral, bacterial or fungal contaminants. It is done on cells and sera before their use in vaccine production, and on the seed stock and the vaccine before and after freeze-drying. Any product that fails this test for sterility is destroyed.

Tests for sterility and freedom from contamination of biological materials may be found in Chapter 1.1.9.

### **c) Safety**

This test is done in rodents in order to detect any nonspecific toxicity associated with the product. The test requires reconstituted vaccine in solvent (mixed contents of five bottles), six guinea-pigs, each weighing 200–250 g; ten unweaned mice (17–22 g, Swiss line or similar).

Vaccine, 0.5 ml, is injected intramuscularly into a hind limb of two guinea-pigs, 0.5 ml into the peritoneal cavity of two guinea-pigs, and 0.1 ml into the peritoneal cavity of six mice. Two guinea-pigs and four mice are kept as uninoculated controls. The animals are observed for 3 weeks. If one guinea-pig or two mice die, the test must be repeated. Dead animals undergo post-mortem examination to ascertain the cause of death. At the end of 3 weeks of observation, all animals are killed for post-mortem examination. All the results are recorded. The vaccine is considered to be satisfactory if, during the first or second test, at least 80% of

animals remain in good health during the period of observation, and no significant post-mortem lesion is found.

**d) Potency and efficacy in small ruminants**

This test requires the following: vaccine reconstituted with normal saline (the mixed contents of five bottles) to provide 100 doses and 0.1 dose/ml; six goats and six sheep, all approximately 1-year old and free from antibodies to rinderpest or PPR; sterile syringes and needles; and pathogenic PPRV previously titrated in goats, diluted with sterile normal saline to provide  $10^3$  of the 50% lethal dose for goats ( $LD_{50}$ ).

Vaccinate two goats and two sheep subcutaneously with 100 doses per animal; vaccinate two goats and two sheep subcutaneously with 0.1 dose per animal; keep the remaining animals as in-contact controls. The animals are observed and temperature measurements are recorded daily for 3 weeks. At the end of this period, blood is taken from all animals for the preparation of sera. All animals are challenged by subcutaneous injection of a 1 ml suspension of pathogenic PPRV ( $10^3 LD_{50}$  per animal). The animals are observed and their body temperature measurements are recorded daily for 2 weeks.

The vaccine is considered to be satisfactory if all vaccinated animals resist the challenge infection, while at least half of the in-contact controls develop signs of PPR. The serum neutralisation test must be positive for PPR antibody (in serum diluted at least 1/10) in vaccinated animals only in samples taken 3 weeks after vaccination. If any of the controls are also positive, the experiment must be repeated using another batch of pathogenic PPRV. The batch of vaccine is destroyed if vaccinated animals react to the virulent challenge.

**o Titration of neutralising PPR antibody**

This test requires the following: cell suspensions at 600,000/ml; 96-well cell culture plates; sera to be titrated (inactivated by heating to 56°C for 30 minutes); complete cell culture medium; PPRV diluted to give 1000, 100, 10 and 1  $TCID_{50}$ /ml.

Dilute the sera at 1/5, then make a twofold dilution in cell culture medium. Mix 100  $\mu$ l of virus at 1000  $TCID_{50}$ /ml (to give 100  $TCID_{50}$  in each well) and 100  $\mu$ l of a given dilution of serum (using six wells per dilution) in the wells of the cell culture plate. Arrange a series of control wells for virus and uninfected cells as follows: six wells with 100  $TCID_{50}$  (100  $\mu$ l) per well; six wells with 10  $TCID_{50}$  (100  $\mu$ l) per well; six wells with 1  $TCID_{50}$  (100  $\mu$ l) per well; six wells with 0.1  $TCID_{50}$  (100  $\mu$ l) per well; and six wells with 200  $\mu$ l of virus-free culture (control cells) per well.

Make the wells containing the virus controls up to 100  $\mu$ l with complete culture medium, and incubate the plates for 1 hour at 37°C. Add 50  $\mu$ l of cell suspension to each well. Incubate the plates at 37°C in the presence of  $CO_2$ . Read the plates after 1 and 2 weeks of incubation. The results should be as follows: 100% CPE in virus control wells of 100 and 10  $TCID_{50}$ , 50% CPE for the 1  $TCID_{50}$  dilution, no CPE for the 0.1  $TCID_{50}$  dilution, no CPE in wells where the virus had been neutralised by serum during the test, and CPE in wells where the virus had not been neutralised by serum during the test.

**e) Duration of immunity**

Duration of immunity is at least 3 years.

**f) Stability**

Freeze-dried vaccine can be kept for at least 2 years at 2–8°C (although storage at –20°C is better), provided it is stored under vacuum and protected from light. Recently, it has been demonstrated that this vaccine, suspended in medium containing trehalose and submitted to the ultra rapid method of dehydration, can resist at 45°C for a period of 14 days with minimal loss of potency (38).

**5. Tests on the final product**

**a) Safety**

See Section C.4.c.

**b) Potency**

See Section C.4.d.

## REFERENCES

1. ANDERSON J. & MCKAY J.A. (1994). The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implication to rinderpest control programmes. *Epidemiol. Infect.*, **112**, 225–234.
2. BERHE G., MINET C., LE GOFF C., BARRETT T., NGANGNOU A., GRILLET C., LIBEAU G., FLEMING M., BLACK D.N. & DIALLO A. (2003). Development of a dual recombinant vaccine to protect small ruminants against peste-des-petits-ruminants virus and capripoxvirus infections. *J. Virol.*, **77**, 1571–1577.
3. BROWN C.C., MARINER J.C. & OLANDER H.J. (1991). An immunohistochemical study of the pneumonia caused by peste des petits ruminants virus. *Vet. Pathol.*, **28**, 166–170.
4. BUNDZA A., AFSHAR A., DUKES T.W., MYERS D.J., DULAC G.C. & BECKER S.A.W.E. (1988). Experimental peste des petits ruminants (goat plague) in goats and sheep. *Can. J. Vet. Res.*, **52**, 46–52.
5. COUACY-HYMAN R.F., HURARD C., GUILLOU J.P., LIBEAU G. & DIALLO A. (2002). Rapid and sensitive detection of peste des petits ruminants virus by a polymerase chain reaction assay. *J. Virol. Methods*, **100**, 17–25.
6. DIALLO A., BARRETT T., BARBRON M., SUBBARAO S.M. & TAYLOR W.P. (1989). Differentiation of rinderpest and peste des petits ruminants viruses using specific cDNA clones. *J. Virol. Methods*, **23**, 127–136.
7. DIALLO A., MINET C., BERHE G., LE GOFF C., BLACK D.N., FLEMING M., BARRETT T., GRILLET C. & LIBEAU G. (2002). Goat immune response to capripox vaccine expressing the hemagglutinin protein of peste des petits ruminants. *Ann. NY Acad. Sci.*, **969**, 88–91.
8. DIALLO A., TAYLOR W.P., LEFEVRE P.C. & PROVOST A. (1989). Atténuation d'une souche de virus de la peste des petits ruminants: candidat pour un vaccin homologue vivant. *Rev. Elev. Med. Vet. Pays Trop.*, **42**, 311–319.
9. DUROJAIYE O.A. (1982). Precipitating antibody in sera of goats naturally affected with peste des petits ruminants. *Trop. Anim. Health Prod.*, **14**, 98–100.
10. DUROJAIYE O.A., OBI T.U. & OJO O. (1983). Virological and serological diagnosis of peste des petits ruminants. *Trop. Vet.*, **1**, 13–17.
11. ELZEIN E.M., HOUSAWI F.M., BASHAREEK Y., GAMEEL A.A., AL-AFALEQ A.I. & ANDERSON E. (2004). Severe PPR infection in gazelles kept under semi-free range conditions. *J. Vet. Med. [B] Infect. Dis. Vet. Public Health*, **51**, 68–71.
12. FORSYTH M.A. & BARRETT T. (1995). Evaluation of polymerase chain reaction for the detection and characterisation of rinderpest and peste de petit ruminants viruses for epidemiological studies. *Virus Res.*, **39**, 151–163.
13. FURLEY C.W., TAYLOR W.P. & OBI T.U. (1987). An outbreak of peste des petits ruminants in a zoological collection. *Vet. Rec.*, **121**, 443–447.
14. GARGADENNEC L. & LALANNE A. (1942). La peste des petits ruminants. *Bull. Serv. Zoo. A.O.F.*, **5**, 15–21.
15. GEORGE A., DHAR P., SREENIVASA B.P., SINGH R.P. & BANDYOPAHYAY S.K. (2006). The M and N genes based simplex and multiplex PCRs are better than the F or H gene based simplex PCR for peste des petits ruminants virus. *Acta. Virol.*, **50**, 217–222.
16. GIBBS E.P.J., TAYLOR W.P., LAWMAN M.J.P. & BRYANT J. (1979). Classification of peste des petits ruminants virus as the fourth member of the genus *Morbillivirus*. *Intervirology*, **11**, 268–274.
17. GOVINDARAJAN R., KOTEESWARAN A., VENUGOPALAN A.T., SHYAM G., SHAGU S., SHAILA, M.S. & RAMACHANDRAN S. (1997). Isolation of peste des petits ruminants virus (PPRV) from an outbreak in Indian Buffalo (*Bubalus bubalus*). *Vet. Rec.*, **141**, 573–574.
18. HAMDY F.M. & DARDIRI A.H. (1976). Response of white-tailed deer to infection with peste des petits ruminants virus. *J. Wildl. Dis.*, **12**, 516–522.

19. KUMAR C.S., RAJ G.D., THANGAVELU A. & SHAILA M.S. (2007). Performance of RT-PCR-ELISA for the detection of peste des petits ruminants virus. *Small Rumin. Res.*, **72**, 200–208.
20. LEFEVRE P.C. & DIALLO A. (1990). Peste des petits ruminants. *Rev. sci. tech. Off. int. Epiz.*, **9**, 951–965.
21. LEFEVRE P.C., DIALLO A., SCHENKEL F., HUSSEIN S. & STAAK G. (1991). Serological evidence of peste des petits ruminants in Jordan. *Vet. Rec.*, **128**, 110.
22. LIBEAU G., DIALLO A., COLAS F. & GUERRE L. (1994). Rapid differential diagnosis of rinderpest and peste des petits ruminants using an immunocapture ELISA. *Vet. Rec.*, **134**, 300–304.
23. LIBEAU G., PREHAUD C., LANCELOT R., COLAS F., GUERRE L., BISHOP D.H.L. & DIALLO A. (1995). Development of a competitive ELISA for detecting antibodies to the peste des petits ruminants virus using a recombinant nucleoprotein. *Res. Vet. Sci.*, **58**, 50–55.
24. MAJIYAGBE K.A., NAWATHE D.R. & ABEGUNDE A. (1984). Rapid diagnosis of PPR infection, application of immuno-electro-osmophoresis (IEOP) technique. *Rev. Elev. Med. Vet. Pays Trop.*, **37**, 11–15.
25. MORNET P., ORUE J., GILBERT Y., THIERY G. & SOW M. (1956). La peste des petits ruminants en Afrique Occidentale Française. Ses rapports avec la peste bovine. *Rev. Elev. Med. Vet. Pays Trop.*, **9**, 313–342.
26. OZKUL A., AKCA Y., ALKAN F., BARRETT T., KARAOGLU T., DAGALP S.B., ANDERSON J., YESILBAG K., COKCALISKAN C., GENÇAY A. & BURGU I. (2002). Prevalence, distribution, and host range of Peste des petits ruminants virus in Turkey. *Emerg. Infect. Dis.*, **8**, 708–712.
27. PERL S., ALEXANDER A., YAKOBSON B., NYSKA A., HARMELIN A., SHEIKHAT N., SHIMSHONY A., DAVIDSON N., ABRAMSON M. & RAPOPORT E. (1994). Peste des petits ruminants (PPR) of sheep in Israel: case report. *Israel J. Vet. Med.*, **49**, 59–62.
28. ROGER F., GUEBRE YESUS M., LIBEAU G., DIALLO YIGEZU L.M. & YILMA, T. (2001). Detection of antibodies of rinderpest and peste des petits ruminants viruses (Paramyxoviridae, Morbillivirus), during a new epizootic disease in Ethiopian camels (*Camelus dromedarius*). *Rev. Med. Vet.*, **152**, 265–268.
29. ROGER F., YIGEZU L.M., HURARD C., LIBEAU G., MEBRATU G.Y., DIALLO A. & FAYE B. (2000). Investigations on a new pathological condition of camels in Ethiopia. *J. Camel Pract. Res.*, **7**, 163–165.
30. ROSSITER P.B., JESSETT D.M. & TAYLOR W.P. (1985). Microneutralisation systems for use with different strains of peste des petits ruminants virus and rinderpest virus. *Trop. Anim. Health Prod.*, **17**(2), 75–81.
31. SALIKI J.T., HOUSE J.A., MEBUS C.A. & DUBOVI E.J. (1994). Comparison of monoclonal antibody-based sandwich enzyme-linked immunosorbent assay and virus isolation for detection of peste des petits ruminants virus in goat tissues and secretions. *J. Clin. Microbiol.*, **32**, 1349–1353.
32. SALIKI J.T., LIBEAU G., HOUSE J.A., MEBUS C.A. & DUBOVI E.J. (1993). Monoclonal antibody-based blocking enzyme-linked immunosorbent assay for specific detection and titration of peste-des-petits ruminants virus antibody in caprine and ovine sera. *J. Clin. Microbiol.*, **31**, 1075–1082.
33. SARAVANAN P., SINGH R.P., BALAMURUGAN V., DHAR P., SREENIVASA B.P., MUTHUCHELVAN D., SEN A., ALEYAS A.G., SINGH R.K. & BANDYOPADHYAY S.K. (2004). Development of an N gene-based PCR-ELISA for detection of Peste-des-petits-ruminants virus in clinical samples. *Acta Virol.*, **48**, 249–255.
34. SHAILA M.S., PURUSHOTHAMAN V., BHASAVAR D., VENUGOPAL K. & VENKATESAN R.A. (1989). Peste des petits ruminants in India. *Vet. Rec.*, **125**, 602.
35. SUMPTION K.J., ARADOM G., LIBEAU G. & WILSMORE A.J. (1998). Detection of peste des petits ruminants antigen in conjunctival smears of goats by indirect immunofluorescence. *Vet. Rec.*, **142**, 421–424.
36. TAYLOR W.P. & ABEGUNDE A. (1975). The isolation of peste des petits ruminants virus from Nigerian sheep and goats. *Res. Vet. Sci.*, **26**, 94–96.
37. TAYLOR W.P., ALBUSAIDY S. & BARRETT T. (1990). The epidemiology of peste des petits ruminants in the Sultanate of Oman. *Vet. Microbiol.*, **22**, 341–352.

38. WORRWALL E.E., LITAMOI J.K., SECK B.M. & AYELET G. (2001). Xerovac: an ultra rapid method for the dehydration and preservation of live attenuated rinderpest and peste des petits ruminants vaccines. *Vaccine*, **19**, 834–839.
39. WOSU L.O. (1991). Haemagglutination test for diagnosis of peste des petits ruminants disease in goats with samples from live animals. *Small Rumin. Res.*, **5**, 169–171.

\*  
\* \*

**NB:** There are OIE Reference Laboratories for Peste des petits ruminants (see Table in Part 3 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list: [www.oie.int](http://www.oie.int)).