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Diagnostic techniques for contagious bovine pleuropneumonia

牛传染性胸膜肺炎诊断技术

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Introduction Introduction

Contagious bovine pleuropneumonia (CBPP) is an infectious disease of bovine animals caused by Mycoplasma mycoides subsp. mycoides (Mmm), also known as cattle lung plague, marked by serous exudative fibrinous inflammation of the interlobular lymphatics of the lung and by serofibrinous pleurisy. The World Organisation for Animal Health lists it as a notifiable animal disease, and the list of class one, two and three animal diseases last revised by China's Ministry of Agriculture and Rural Affairs in 2022 places it among the class one animal diseases.

China developed an effective attenuated vaccine in 1960 and, through wide and dense vaccination combined with slaughter, quarantine inspection and other control measures, saw no further clinical case after 1989 and finally eliminated the disease. In 2011 China

received the WOAH certificate of freedom from CBPP and became an internationally recognised CBPP-free country.

The laboratory detection methods of the document, being pathogen isolation, the polymerase chain reaction (PCR), the complement fixation test (CFT) and the competitive enzyme-linked immunosorbent assay (ELISA), are each applied in the different situations set out in Annex A.

1 Scope

The document describes the epidemiology, clinical signs, pathological changes, sample collection, storage and handling of CBPP, together with the diagnostic methods of pathogen isolation and identification, PCR detection, CFT detection and competitive ELISA.

It applies to the diagnosis, quarantine inspection, surveillance and epidemiological investigation of CBPP.

2 Normative references

Three undated references are cited: GB/T 6682 on water for analytical laboratory use, GB 19489 on general requirements for laboratory biosafety, and NY/T 541 on technical specifications for the collection, storage and transport of veterinary diagnostic samples.

3 Terms and definitions

The document states that it has no terms or definitions that need defining.

4 Abbreviations

CBPP, contagious bovine pleuropneumonia; CFT, complement fixation test; DNA, deoxyribonucleic acid; ELISA, enzyme linked immunosorbent assay; Mmm, Mycoplasma mycoides subsp. mycoides; OD value, optical density; PBS, phosphate buffered saline; PCR, polymerase chain reaction.

5 Laboratory biosafety measures

Once cattle are judged to be suspected cases of CBPP infection, the relevant material is sent to a laboratory approved by the agricultural administrative authority for further pathogen isolation and identification. Laboratory diagnosis of CBPP is carried out under GB 19489 and, following the Detailed Rules on Biosafety Requirements for Experimental Activities with Animal Pathogenic Microorganisms, pathogen isolation and identification are to be carried out in a biosafety level three laboratory. Sample collection, storage and transport follow NY/T 541. Water used to prepare the reagents of the document shall meet GB/T 6682. A laboratory that has not obtained the approval of the agricultural administrative

authority shall not carry out isolation and culture of the pathogen.

6 Clinical diagnosis

6.1 Epidemiological features. Infected cattle are the chief source of infection. Inhalation of droplets from infected cattle by healthy cattle after direct contact is the chief route of transmission, the droplets being the vehicle. The disease may also be transmitted vertically through the semen and embryos of infected cattle. Under natural conditions yellow cattle, dairy cattle, yaks and dzo are the most susceptible, buffalo are less so; deer and antelope are also fairly susceptible, and goats and sheep may be infected.

6.2 Clinical signs. The incubation period averages 7 d to 28 d, being as short as 7 d and as long as several months. By course the disease is divided into acute, subacute and chronic forms. In the acute form the signs are marked, typical and characteristic: the body temperature rises to 40 °C to 42 °C in a persistent fever; breathing is laboured and shallow, and abdominal; coughing grows frequent; the chest is painful so the animal is unwilling to move or lie down; expiration is long and inspiration short, with the neck stretched out and gasping, often with groaning; there is a serous or purulent nasal discharge; rumination and milk production stop and appetite is lost; the visible mucous membranes are cyanotic and the coat rough and disordered; percussion of the chest may give a dull or flat area on the affected side; auscultation over the affected part gives moist rales, with the vesicular sound weakened or lost and replaced by bronchial breathing and at times a friction sound. In the late stage the heart weakens and the pulse becomes thready and fast, reaching 80 to 120 beats per minute. Death generally follows in 8 d to 10 d, the whole course lasting about 15 d to 30 d. The subacute form resembles the acute but is somewhat milder and runs a rather longer course; when the temperature rises to 40 °C breathing is markedly laboured, the animal stands with its neck stretched and head lowered and moves with difficulty, refuses feed and is weak in spirit, and the prognosis is poor. The chronic form mostly follows from the acute or subacute; the temperature is irregular with transient fever, often resembling bovine tuberculosis; the animal wastes, digestion is disordered and appetite fluctuates. Given good nursing and proper treatment such an animal may recover gradually and become a carrier; where the lesions are extensive it grows weaker by the day and the prognosis is poor.

6.3 Pathological changes. The characteristic changes are chiefly in the lung and the thoracic cavity, the typical change being a marbled appearance of the cut surface of the lung and serofibrinous pleuropneumonia. Early in the course the picture is lobular bronchopneumonia, and at the middle stage a typical serofibrinous pleuropneumonia, mostly on the right side and at times on both. The lesions are most often in the diaphragmatic lobe of the lung, and may also occur in the cardiac or the caudal lobe. The pleura commonly shows haemorrhage and thickening and adheres to the diseased part of the lung. The pulmonary pleura and the pericardium carry fibrinous deposits on their

surface.

6.4 Judgement of the result. Where an animal shows some or all of the clinical signs and pathological changes above, it may be judged a suspected case of CBPP. Confirmation calls for further sampling and laboratory diagnosis.

7 Sample collection, storage and handling

7.1 Reagents and materials: medical protective clothing; sterilised scissors and forceps; sterile cotton swabs; disposable syringes of various sizes, sterile collection bags and collection tubes; PBS solution at pH 7.4, prepared under B.1 of Annex B; and PBS solution containing penicillin and streptomycin, prepared under B.2.

7.2 Sample collection. Blood samples: 10 mL of blood is drawn aseptically with a syringe and serum separated after clotting, or anticoagulated blood is collected and plasma separated by centrifugation; anticoagulated blood or plasma may be used for isolation of Mmm and for PCR, and serum for detection of antibody to Mmm. Tissue samples: a block of lung about 2 cm square is collected aseptically from an animal that has died or been killed for examination, taken at the boundary between diseased and normal tissue, together with part or all of the hilar lymph nodes, and placed sealed in a sterile collection bag or other sterilised container for isolation of Mmm and for PCR; 3 mL to 5 mL of thoracic effusion, bronchial lavage fluid, pericardial fluid or, where there are signs of arthritis, joint fluid, is collected aseptically into sterile collection tubes for the same purposes. Nasal swab samples: a sterilised cotton swab is put into the nasal cavity and turned to wipe the mucosa three to five times; once mucus adheres, the swab is placed in a sampling tube holding 2 mL of PBS solution at pH 7.4 containing penicillin and streptomycin, capped and sealed, for isolation of Mmm and for PCR.

7.3 Storage. Samples are placed in an insulated box as soon as possible with dry ice or pre-cooled ice packs, sealed, kept in an unbroken cold chain and sent to the laboratory within 24 h. Samples awaiting examination are handled as soon as possible and held for not more than 24 h at 4 °C, or frozen at -20 °C for not more than 6 months. For long storage they should be held at -70 °C. Repeated freezing and thawing is to be avoided as far as possible.

7.4 Handling. Serum samples are centrifuged at 2 000 r/min for 5 min and the supernatant taken. Tissue samples: about 2 g of the lung or lymph node sample is broken up cold in a tissue grinder, 10 mL of PBS solution containing penicillin and streptomycin added to make a tissue suspension, the suspension shaken and mixed thoroughly and centrifuged at 6 000 r/min for 5 min at 4 °C, and the supernatant taken. Swab samples are mixed on a vortex shaker and centrifuged at 6 000 r/min for 15 min at 4 °C and the supernatant taken. Body fluid samples, being thoracic effusion, bronchial lavage fluid, pericardial fluid and joint fluid, are centrifuged at 6 000 r/min for 5 min at 4 °C and the supernatant taken.