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Diagnosis of Contagious Caprine Pleuropneumoniae

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INTRODUCTION

Contagious caprine pleuropneumonia (CCPP) is a highly fatal disease that occurs in goats. Outbreaks have been reported in sheep and captive wildlife. This disease attacks the lungs and the mucosal membranes that line the thoracic cavity causing fever and respiratory signs such as laboured or rapid respiration, cough and nasal discharges. The factors like extremes of age, stress and intercurrent infection predispose the animal to tissue invasion by bacteria. The disease is caused by *Mycoplasma capricolum capri pneumoniae*. Morbidity can be 100%, and mortality 60%–100%.

Transmission of the disease occurs through direct contact between an infected and a susceptible animal which becomes infected by inhaling droplets disseminated by coughing. Since some animals can carry the disease without showing signs of illness, controlling the spread is more difficult.

The disease is manifested by weakness, anorexia, cough, hyperpnoea, and nasal discharge accompanied by fever (104.5°–106°F) are often found. Exercise intolerance progresses to respiratory distress, with open-mouth breathing and frothy salivation. A septicemic form of the disease can also occur without specific respiratory tract involvement.

Lesions involve an excess of straw-colored pleural exudate and acute fibrinous pneumonia. There can be unilateral lung consolidation.

Diagnosis

Direct microscopy

Leishman- or Giemsa-stained blood smears may be used to demonstrate pleomorphism and weak staining of *Mycoplasma*.

Cultural characters and biochemical differentiation

Mycoplasma require enriched media containing animal protein, a sterol component and a source of DNA or adenine dinucleotide.

Commercially available mycoplasma agar or broth media (often heart infusions) are supplemented with 20% horse serum and yeast extract providing amino acids and vitamins. In addition, penicillin is used to inhibit Gram - positive bacteria, and thallous acetate is incorporated to inhibit Gram - negative bacteria and fungi. Media are buffered at pH 7.3 to 7.8 for Mycoplasma species.

Inoculation of culture media

For routine isolation of mycoplasmas, the specimen should be inoculated into two broths and onto two plates of agar. The inoculation technique will vary according to the nature of the specimen:

1. Fluid materials such as foetal fluids and exudates can be inoculated directly into broth medium and spread over the surface of the agar medium.
2. Some specimens such as semen, joint fluids and tissues may contain inhibitors for mycoplasmas. Both undiluted specimen and 10-fold dilutions in mycoplasmal broth (up to 10−6) should be cultured.
3. With tissues, such as pieces of lung, a freshly cut surface on a block of the tissue can be moved across the surface of an agar plate to inoculate it.

The inoculated agar plates are incubated in a humid atmosphere at 37°C. It is advisable to inoculate and incubate duplicate plates, one aerobically and one under 5% CO2 and 95% N2. A candle jar may be satisfactory. The plates should be examined after 48 and 96 hours' incubation, for the characteristic mycoplasmal 'fried-egg' microcolonies. The cultures can be regarded as negative if no microcolonies are seen after 14 days' incubation.

Obtaining a pure culture

A pure culture of the isolated Mycoplasma should be obtained before carrying out identification techniques. The microcolonies grow into the agar and it is difficult to obtain cells from the colonies on an inoculation loop, so different methods have to be used for subculture. An agar plate should be selected with well separated colonies. Individual colonies are removed by cutting out a small block of agar, containing the colony of interest, with a sterile scalpel. The block is transferred to a tube with 2–3 mL of broth and incubated at 37°C for 48 hours or longer. The broth culture, when showing turbidity, is taken into a sterile syringe and passed through a 0.45 µm membrane filter. The filtrate is diluted with fresh broth, 1 : 10 and 1 : 100, and a loopful of each dilution is inoculated onto agar medium and the plates incubated. This procedure should be repeated three times.

Another method is to take a block of agar containing one colony and place it colony-side downwards on an agar plate. The agar block is pushed back and forth over the agar surface and the inoculated plate incubated for 48–72 hours. This procedure is repeated twice more.

Once a pure culture has been obtained, normal subculturing can be carried out using similar techniques, either from agar-to-agar or agar-broth-agar.

Colonial morphology

When examined microscopically at low magnification, unstained microcolonies of *Mycoplasma* species are 0.1 to 0.6 mm in diameter and have a 'fried-egg' appearance.

Dienes stain facilitates recognition of microcolonies by staining the central zone dark blue and the peripheral zone a lighter blue.

Microcolonies of Mycoplasma species require differentiation from colonies of bacterial L-forms. Because bacterial L-forms lack a rigid cell wall, they assume shapes resembling mycoplasmas. However, L-forms can revert to their normal bacterial shape and produce cell walls and typical bacterial colonies when subcultured on non-inhibitory media.

Immunological tests, using specific antisera produced against each pathogenic species, can be used for specific identification. Growth inhibition tests, in which filter paper discs containing specific antisera are placed on an

agar surface seeded with the mycoplasma under test, are used for diagnosis. A zone of growth inhibition up 8 mm wide develops around the disc containing homologous antiserum. Fluorescent antibody staining of individual microcolonies can also be used for identification. Complement fixation tests for the major mycoplasmal diseases of ruminants are used for certification when animals are traded internationally. Tests based on ELISA are being developed for the diagnosis of economically important mycoplasmal diseases.

Molecular tests targeting DNA can be used for identification of bacteria. PCR tests for differentiation of most mycoplasmas pathogenic for animals have been developed

Strain Typing

Typing of Mycoplasma organisms is frequently carried out by enzymatic digestion of extracted DNA, followed by pulsed-field gel electrophoresis (PFGE). Variable number tandem repeat (VNTR) analysis and random amplified polymorphic DNA techniques may also be used.