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IZS TERAMO
ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'ABRUZZO E DEL MOLISE "G. CAPORALE"

VETERINARY SCIENCES RESEARCH INSTITUTE

PRACTICAL GUIDE TO THE LABORATORY CULTURE AND ISOLATION OF MYCOPLASMA MYCOIDES SUBSPECIES MYCOIDES SMALL COLONIES (MMMSC)

Introduction

Mycoplasma mycoides subspecies mycoides small colonies (*MmmSC*) is the causative agent for Contagious Bovine Pleuropneumonia (CBPP). CBPP is a severe respiratory disease of cattle that causes major economic loses in many parts of Africa. *MmmSC* is fastidious and hence requires careful laboratory handling and specialized media for successful isolation and culture. Culturing helps in confirmation of an infection.

Objective

To demonstrate the laboratory procedure for the isolation and culture of *MmmSC* from clinical samples.

Principle

MmmSC requires enriched media called Newing's Tryptose broth and Newing's Tryptose agar containing pig or horse serum and other growth factors to grow. When cultured, it grows and typically appears as small colonies with a light outer zone and a dense raised centre, often described as the "fried egg appearance" when viewed under the microscope.

Materials Required

- Newing's Tryptose broth
- Newings tryptose agar
- Sterile pipettes
- Sterile 15ml centrifuge tubes
- Sterile inoculating loops
- Incubator set at 370C.
- Clinical sample (e.g lung tissue or pleural fluid)
- Biosafety cabinet
- Personal protective equipment

Procedure

1.**Tryptose broth dispensing**

Aliquote 2.7ml of the tryptose broth into sterile centrifuge tubes preferably leave them in the incubator at 37^0C overnight.

2.Sample preparation

Collect the sample from the cattle suspected to have CBPP or retrieve the sample if stored in the laboratory. If it is tissue sample, homogenize it in sterile broth.

3.Inoculation into Tryptose broth

Transfer 0.3ml of the pleural fluid into 2.7ml of tryptose broth using a pipette and mix for a moment, continue with a serial dilution up to 10^{-4} by transferring 0.3ml and discarding 0.3ml from the last tube.

For the tissue sample, pick a small amount of the homogenized sample into 0.3ml of the broth and perform serial dilutions as for the pleural fluid sample.

4.Subculturing on Tryptose agar

In a biosafety cabinet, streak 20ul of each dilution of the broth culture onto tryptose agar plates using a sterile inoculating loop.

5.Incubation

Incubate the broth and agar cultures at 37^OC for 3-7 days or sometimes longer.

6.Culture observation and expected results

Daily examine the broth culture for colour change from red to yellow and the plates for the “fried egg” colony morphology typical of Mmm.

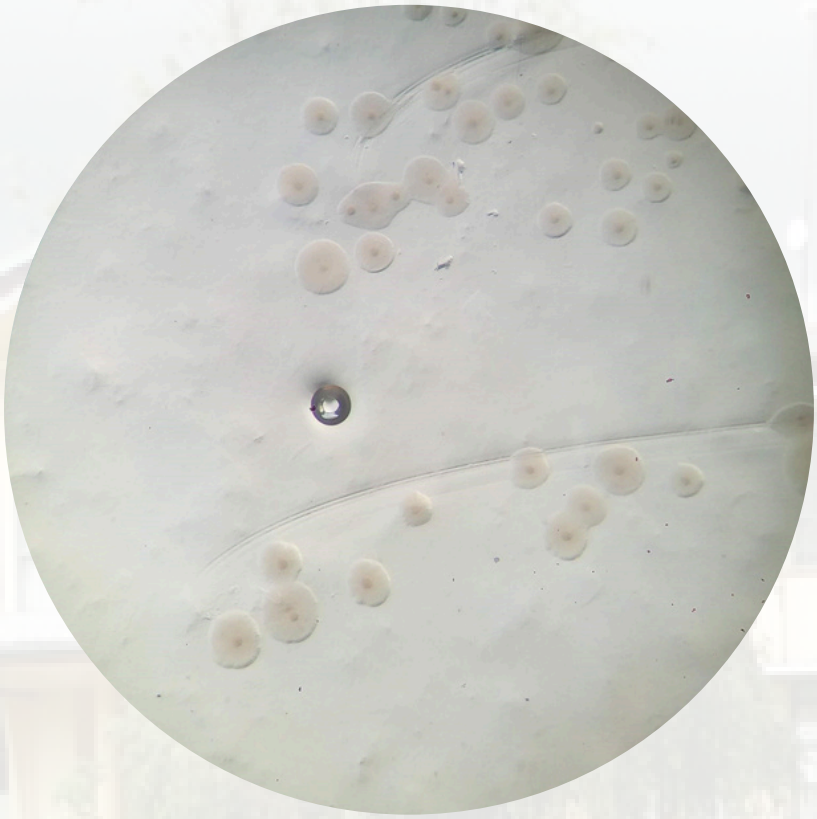

Fig 1: showing a microscope field of view with Mmm colonies

Conclusion

Laboratory culture and identification of *Mycoplasma mycoides* subspecies *mycoides* provide an essential confirmation of CBPP in support of abattoir observations. During meat inspection, suspected lesions can be identified and appropriate samples collected for laboratory analysis. Effective collaboration between abattoir inspectors and laboratory personnel will enhance early detection, accurate diagnosis, and timely reporting, ultimately supporting the efforts to control and prevent the spread of CBPP.

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