AUSTRALIAN STANDARD DIAGNOSTIC TECHNIQUES FOR ANIMAL DISEASES

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Bovine Trichomoniasis

Microbiology

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Contents

1. Introduction 3
2. *Tritrichomonas foetus* 3
3. Bulls 3
   3.1. Collection of sample from bull 3
   3.2. Forwarding of samples to laboratory 3
   3.3. Laboratory procedures 3
   3.4. Selective medium for *Tritrichomonas foetus* 4
4. Cows and heifers 4
5. References 4

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1. Introduction

Bovine Trichomoniasis is a disease of the reproductive tract caused by the protozoan *Tritrichomonas foetus*. Today, trichomoniasis is seldom seen in cattle in Southern Australia but it persists as a sometimes intractable problem in extensively managed herds in Northern Australia. The characteristic signs of infection in breeding herds are infertility, early foetal death and a marked increase in the interval between calvings. Infection is localised to the penile and preputial mucosa in bulls and in females, the vagina, cervix and uterus.

Diagnosis of bovine trichomoniasis requires the demonstration of the flagellate protozoan, *T. foetus* by direct microscopic examination of genital secretions or exudates in the field or more commonly, after laboratory culture of specimens. Improved sampling and cultural procedures have greatly assisted diagnosis, increasing the numbers of the protozoa obtained, thus facilitating and simplifying microscopic examination.

Three serotypes of *T. foetus* exist in Australia. They are designated, Belfast, Manley and Brisbane (Clark *et al.*, 1971).

2. *Tritrichomonas foetus*

T. foetus in culture is pyriform or round in shape but narrower forms are more common in material from cattle. Length varies from 5 to 18 µm and breadth is about 7 µm at the greatest width. There is a large nucleus placed anteriorly and an axostyle which projects a short distance beyond the posterior extremity. An undulating membrane is present along one of the lateral margins of the cell. There are three anterior flagellae and one posterior flagellum which arises from the outer edge of the undulating membrane.

The flagellae and undulating membrane of *T. foetus* permit the organism to be freely motile in preputial secretions and less commonly in vaginal secretions.

Trichomonads exhibit a jerky movement in wet preparations and when stationary, can still be identified by the motion of the undulating membrane and flagella.

The typical irregular, jerky movement and general morphology of the organism can be seen at a microscopic magnification of x80–x100 but higher magnification (x300–x400) is required to view the flagellae and undulating membrane.

3. Bulls

3.1. Collection of Sample from Bull

Put bull in crush and fasten nearest hind leg with leg rope. Introduce pipette to full length of preputial cavity; squeeze bulb and collect material in ventral fornix and then suck up material along dorsal surfaces of penis and surrounding preputial mucosa for 30 s to one minute, controlling placement of the flat bevelled end of pipette through wall of prepuce by hand. The flat bevelled end of the pipette is directed onto these surfaces during collection and orientation is assisted by observing the bend of the pipette. Samples will vary in consistency from liquid (0.5–2 mL) to thick sticky cellular material. Rinse out the pipette by carefully sucking up 4 mL of sterile physiological saline from a screw-capped sample bottle and allow this to drain back into the bottle. The label on this bottle should be marked clearly with identification of bull, and time and date of collection.

3.1.1. Materials Required

3.1.1.1. Plastic pipettes

Made from perspex, polystyrene or polypropylene (exernal diameter 1 cm; internal diameter 0.6 cm), 60 cm in length, straight except for a bend at the end that is held by the operator during collection; the other end that contacts preputial surfaces is bevelled.

N.B. After use, thoroughly clean and rinse pipettes with warm water and immerse overnight in tap water containing 30 mL of sodium hypochlorite (NaOCl) per litre (use household products with about 1% free chlorine). Then rinse pipettes out thoroughly with tap water to remove traces of chlorine and wrap in clean paper or store in suitable containers. Sterilise the bulbs by boiling for 10 min.

3.1.1.2. Rubber bulbs

Firm rubber bulbs to put on pipettes, capacity 85 mL (3 oz.).

3.1.1.3. Sterile labelled screw-capped containers

These should be 20 mL capacity and each containing 4 mL of sterile physiological saline.

3.2. Forwarding of Samples to Laboratory

Allow diluted sample in labelled container to stand for 10 min and then collect 0.5–1 mL of sedimented cells from bottom of container with sterile pasteur pipette and inoculate a labelled screw-capped bottle containing selective medium for *T.* foetus (see 3.4.). Clearly mark the latter bottle with identification of bull, and time and date of collection. Store and transport inoculated medium at room temperature and deliver to laboratory within 48 hours of collection. Avoid direct sunlight, freezing or excessively hot temperatures (>37°C).

3.3. Laboratory Procedures

Inoculated medium received at the laboratory should be incubated at 37°C for four days prior to microscopic examination. To examine, collect 0.2 mL of medium from the bottom of the bottle with a pasteur pipette and examine on a glass slide by microscopy (x80) using bright field illumination.

T. foetus is identified by the characteristic motility and morphology of the organism. Examine the specimen on the slide for at least five minutes.

3.4. Selective Medium for Tritrichomonas foetus

The medium is the modified Plastridge medium described by Sutherland *et al.* (1953).

3.4.1. Formulation

A base is prepared to which is added equal quantities of sterile inactivated ox serum. The composition of the base is:

Liver infusion	1 L
Bacto peptone (Difco)	10 g
Bacto Agar (Difco)	3 g

Liver infusion can be prepared from fresh bovine liver or Bacto Liver (Difco) may be used. (Difco Laboratories, Detroit, Michigan, USA. Australian Distributor: FSE, 47–49 Overseas Drive, Noble Park, Vic. 3174. Tel. (03) 795 0077; Fax (03) 790 1900.

If using fresh liver, mince 500 g of fresh beef liver into 1 L of glass distilled water, stand in cold room overnight, skim off fat and heat to 45°C and hold at 45–50°C for one hour. Then boil for 30 min without stirring. Then lift out coagulum and filter infusion through glass wool or paper. Add glass distilled water to return volume to 1 L. If using Bacto Liver, add 122 g to 1 L and follow as above for fresh liver.

To liver infusion prepared by either method, add Bacto peptone, dissolve by steaming, adjust to pH 7.4 and steam for 15 min. Cool to 20°C and filter through paper, add agar, steam to dissolve and distribute to 8 mL quantities in 20 mL sterile screw-cap universal containers. Autoclave at 121°C for 15 min. Base can be stored in cupboard for months prior to use.

N.B. Addition of 12 mL of 10% sodium hydroxide (NaOH) to each litre before addition of peptone will bring pH to about 7.4 after steaming.

3.4.2. Addition of Ox Serum and Antibiotics

Serum and antibiotics are added to base prior to use. (Addition can be carried out several days prior to use). The base is dissolved by heating in boiling water bath (or steamer), cooled to 40°C and equal quantities of inactivated sterile ox serum added and mixed. Penicillin and Streptomycin are added to medium to a final level of 1000 units/mL of each antibiotic. Use sodium salt of Penicillin G crystalline. Fungizone (Squibb) is added to the medium to a final level of 2.5–5 µg/mL. Keep medium in the refrigerator (4°C) until required.

4. Cows and Heifers

Samples of vaginal mucus are aspirated from the anterior of the vagina using glass or plastic pipettes and a long rubber tube. Mucus should be collected preferably during the latter half of the oestrus cycle. Use a long piece of wire (previously heated in flame to sterilise) to push cotton wool plug through the collection tube and so express mucus. Inoculate a labelled screw-capped bottle containing selective medium for *T. foetus* (see 3.4.) with 0.25–0.5 mL of vaginal mucus, and clearly mark the bottle with identification of the animal and time and date of collection. Store and transport inoculated medium at room temperature and deliver to laboratory within 48 hours of collection. Avoid direct sunlight, freezing or excessively hot temperatures (>37°C). Inoculated medium received at the laboratory should be incubated at 37°C and examined microscopically at four and seven days. To examine, collect 0.2 mL from the bottom of the bottle with a pasteur pipette and examine on a glass slide by microscopy (x80) using bright field illumination. *T. foetus* is identified by the characteristic motility and morphology of the organism.

5. References

Clark, B.L., White, M.B., and Banfield J.C. (1971). Diagnosis of *Tritrichomonas foetus* infection in bulls. *Australian Veterinary Journal* **47**, 181-3.

Sutherland, A.K., Simmons, G.C. and Bell, A.T. (1953). An outbreak of Bovine Trichomoniasis Part 2: Diagnosis. *Australian Veterinary Journal* **29**, 67-9.