

RESEARCH COMMUNICATION

AN EFFICIENT MEDIUM FOR THE ISOLATION OF *TRICHOMONAS FOETUS*

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ABSTRACT

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Trials were conducted to compare a modification of a commercial medium with the 2 media currently used at the Veterinary Research Institute, Onderstepoort, for the isolation of *Trichomonas foetus*. An antifungal agent was also added to the medium. Results obtained proved the modified medium to be more reliable for the isolation of *T. foetus* than the other 2, and the addition of antifungal more efficient in the control of contaminants.

INTRODUCTION

Trichomonas foetus (*T. foetus*) is a flagellate protozoan which causes a venereal disease in cattle characterized by early embryonic death with irregular oestrus cycles and infertility, a low percentage of abortions and pyometra (Parsonson, Clark & Dufty, 1974; Lowe, 1978; BonDurant, 1985). Infected bulls show either no clinical signs (Kimsey, 1986) or only a transient balanoposthitis (Jubb, Kennedy & Palmer, 1985). However, they carry the organism in the fornx of the prepuce (sheath) and the mucosa of the penis (Parsonson *et al.*, 1974; Lowe, 1978), and can infect susceptible females during coitus.

Bovine trichomoniasis is a highly prevalent disease in South Africa. *T. foetus* was isolated from 89 (35,46 %) out of 251 herds, where the bulls were examined by preputial washes processed at the Veterinary Research Institute, Onderstepoort, over a period of 30 months (Ribeiro, unpublished data, 1989). An infected herd will show a lower than normal conception rate of between 40-70 % (Clark, Parsonson, White, Banfield & Young, 1974; BonDurant, 1985), thus illustrating the economic impact of this disease.

The only valid method of establishing a diagnosis of trichomoniasis is to isolate and identify the causative agent, *T. foetus* (Clark, White & Banfield, 1971). Although the success rate of isolation depends on the suitability of the sampling technique, conditions of transport and time lapse prior to the processing of the sample, culturing methods and media used can also affect the recovery of *T. foetus* (Reece, Dennett & Johnson, 1983). In the author's experience, only 30 % of infected sheathwashes can be identified by using direct examination alone, and therefore a reliable culture method is essential. This paper reports on the results obtained with a new medium and compares its reliability with that of the 2 media currently in use. An antifungal agent was also used for the control of contamination.

MATERIALS AND METHODS

Media

A *Trichomonas* medium (TR), based on Oxoid *Trichomonas* medium¹ for the detection of *Trichomonas vaginalis* and *Candida* species, was prepared. It contains liver digest (25 g/l), sodium chloride (6,5 g/l), dextrose (5 g/l) and agar No. 1 (1 g/l). To this was added 10 % horse serum, inactivated for 30 min at 60 °C. The pH of the medium was then ad-

justed to 7,2. The 2 media currently used at Onderstepoort are glucose medium (GL) and liquid paraffin medium (LP), prepared as described by Pefanis, Herr, Venter, Kruger, Queiroga & Amaral (1988). All media were dispensed in 12 ml quantities into 20 ml McCartney bottles and stored at 4 °C.

Antibiotics

Antibiotics currently added to the media at Onderstepoort are sodium benzylpenicillin² (1 660 i.u./ml) and streptomycin sulphate³ (1,66 mg/ml). These were included in all the media, and amphotericin B⁴ (5 µg/ml) was added to half of each batch of medium to curb the growth of contaminating yeasts.

Specimens

Eight hundred and thirty-two sheathwash samples from 85 herds, submitted by private owners or veterinarians for the diagnosis of trichomoniasis, were used for this investigation. Specimens were collected in 50 ml phosphate buffered saline (PBS; pH 7,2), packed on ice and transported to the laboratory under protection from direct sunlight. Also used were 182 vaginal mucus samples collected over a period of time from a herd known to be infected with *T. foetus*.

Direct examination and culture techniques

Sheathwashes were centrifuged for 10 min at 1 200 g, the supernatant decanted and the sediment resuspended in the final 1 ml of supernatant. One drop of this suspension or 1 drop of vaginal mucus was examined using a phase contrast microscope at 100 × magnification. Four drops of the resuspended sediment or vaginal mucus were inoculated into 12 ml of each of the 3 media. The cultures were incubated at 32 °C and examined after 48, 96 and 144 h. One drop of each culture was examined by direct microscopic examination.

Interpretation

The finding of at least 1 *T. foetus* organism, either on direct examination or in culture, was considered a positive result.

RESULTS AND DISCUSSION

Of the 832 sheathwash samples examined, 115 (13,82 %) were found positive for *T. foetus*. Of these, as only 23 (20 %) were positive on direct

¹ Code CM161, Oxoid Ltd, Basingstoke, Hampshire, England

² Novopen, Novo Industries (Pharmaceuticals) (Pty) Ltd

³ Novo-Strep, Novo Industries (Pharmaceuticals) (Pty) Ltd

⁴ Fungizone, E. R. Squibb & Sons, Inc., Princeton, USA

examination, as many as 80 % of all infected bulls could remain undiagnosed, if culture is not attempted. All 23 samples were positive on culture in the TR medium, but 1 sample (4,35 %) failed to yield any *T. foetus* in either the GL or LP media. The remaining 92 positive samples were all positive on the TR medium, 2 (2,17 %) did not grow in the GL medium and 3 (3,26 %) were negative on the LP medium. Thus, out of 115 animals found infected with *T. foetus*, the TR medium was successful in identifying 100 % of the infections, the GL medium detected 113 (98,3 %) infected samples, and the LP medium detected 112 (97,4 %) animals with *T. foetus*.

Of the 182 vaginal mucus samples examined, 111 (61,0 %) were found to be infected with *T. foetus*. Of these, 82 (73,9 %) were positive on direct examination. The high percentage of samples found positive on direct examination may be due to the fact that the animals were all recently infected heifers and that the samples were all processed within 1 h of collection. All the samples which were found positive for *T. foetus*, including the 82 that were positive on direct examination, grew on culture in the TR medium. Of the 82, 1 sample (1,22 %) failed to grow on either the GL or LP media. Of the remaining 29 samples which were positive on culture only, 8 (27,6 %) did not grow in the GL medium and 13 (44,8 %) were negative on the LP medium. The TR medium thus identified 100 % of the animals which were found infected, the GL medium detected 102/111 (91,9 %) infected samples, and the LP medium detected 97/111 (87,4 %) samples with *T. foetus*.

Growth of *T. foetus* was consistently better in the TR medium than in either of the other 2 media. Also the organisms multiplied faster, thus allowing for an earlier detection of infection in samples that were negative on direct examination. Thus, of the 111 vaginal mucus samples that were found to be positive, 107 (96,4 %) were positive on the TR medium after 96 h incubation, compared to 90 (88,2 %) out of 102 positive samples in the GL medium and 81 (83,5 %) out of 97 in the LP medium after the same period of time. The incorporation of agar into the TR medium may account for the more prolific growth of *T. foetus*, as it leads to reduced oxygen tension in the medium (Oxoid, 1982).

Many of the samples were moderately contaminated with yeasts. When penicillin and streptomycin only were used, the yeasts multiplied to such an extent as to make the detection of *Trichomonas* organisms difficult. *T. foetus* did not multiply as well in these samples as in those cultivated in media to which amphotericin B had been added. The tricho-

monads also survived for longer periods of time in the media containing the antifungal agent.

A statistical analysis of the results of this investigation indicated that the TR medium was significantly more efficient (chi-square test; $P = 0,05$) than either the GL or LP media in detecting *T. foetus* in vaginal mucus samples. With sheathwash samples, the difference in results was not statistically significant, but the improved rate of growth of *T. foetus* in the TR medium may be advantageous in practice. There are indications that the addition of amphotericin B could also lead to better growth of *T. foetus* in the medium. The combined benefits of these 2 modifications would, therefore, have a positive effect in improving the diagnosis of trichomoniasis in cattle.

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