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RESEARCH COMMUNICATION

THE USE OF FILTER PAPER DISCS IMPREGNATED WITH THIONIN ACETATE, BASIC FUCHSIN AND THIONIN BLUE IN THE IDENTIFICATION OF *BRUCELLA* SPECIES

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ABSTRACT

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Filter paper discs impregnated with solutions containing 0,25, 0,5 and 1 mg/mℓ of thionin acetate, 0,75 and 1,5 mg/mℓ of basic fuchsin and 0,5 mg/mℓ of thionin blue were used in the typing of *Brucella* species. All the strains used reacted as expected, proving this new technique to be reliable in the identification of *Brucella* species. The method is less expensive and the results easier to interpret than those obtained with methods previously used.

INTRODUCTION

The genus *Brucella* comprises closely related Gram-negative bacteria currently classified into 6 species: *B. abortus, B. melitensis, B. ovis, B. canis, B. suis* and *B. neotomae* (Alton, Jones, Angus & Verger, 1988). *B. abortus, B. melitensis* and *B. suis* are further classified into several biotypes (Corbel, Gill & Thomas, 1983; Corbel & Brinley-Morgan, 1984; Alton *et al.*, 1988). All these species have a world-wide distribution, with the exception of *B. neotomae* and possibly *B. canis* (Corbel *et al.*, 1983; Joint FAO/WHO expert committee on brucellosis, 1986). *B. abortus* and *B. ovis* are the species most commonly found in the Republic of South Africa (Herr, unpublished annual reports 1982–1989).

Even though the various *Brucella* species differ in the frequency with which they infect particular host animals and in their degree of host specificity, most *Brucella* species can cause natural infection in different animals and man (Corbel *et al.*, 1983; Corbel & Brinley-Morgan, 1984). *B. ovis* is an exception and occurs naturally only in sheep (Alton *et al.*, 1988). Man usually becomes infected by contact with infected animals and contaminated materials, or by consuming contaminated food of animal origin (Fensterbank, 1986; Joint FAO/WHO, 1986). Consequently, brucellosis is a public health problem besides being a serious economic problem.

The bacteriological isolation and identification of the various *Brucella* species and biotypes is indispensable for obtaining an accurate evaluation of the epidemiological status of herds, communities and countries (Fensterbank, 1986; Joint FAO/WHO, 1986). Also significant is the need to differentiate virulent field strains from the strains used in vaccines, such as *B. abortus* strain 19 (S19) and *B. melitensis* Rev. 1 (Corbel *et al.*, 1983; Joint FAO/WHO, 1986). Among the tests used for the differentiation of *Brucella* species are tolerance to dyes, such as thionin acetate, basic fuchsin and thionin blue (Alton, Jones & Pietz, 1975; Corbel *et al.*, 1983; Corbel & Brinley-Morgan, 1984; Joint FAO/WHO, 1986). These tests are usually done by the use of dye sensitivity test media, which are prepared by the addition of the relevant dye to a basal medium (Corbel *et al.*, 1983). Nevertheless, both the preparation of the media and the interpretation of the results are subject to error. It was therefore decided to investigate the possibility of using dye-containing discs for the tests, as these would simplify the typing procedures, reduce costs and give results of easier interpretation and increased reliability.

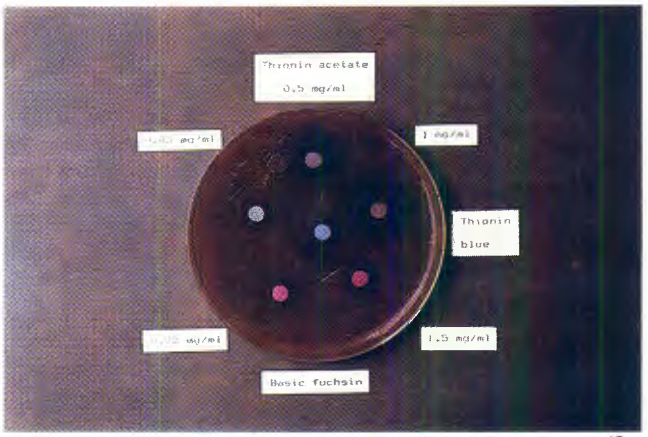

FIG. 1. *Brucella abortus* biotype 2 inhibited by thionin acetate (3 concentrations), basic fuchsin (2 concentrations) and thionin blue discs on BTA

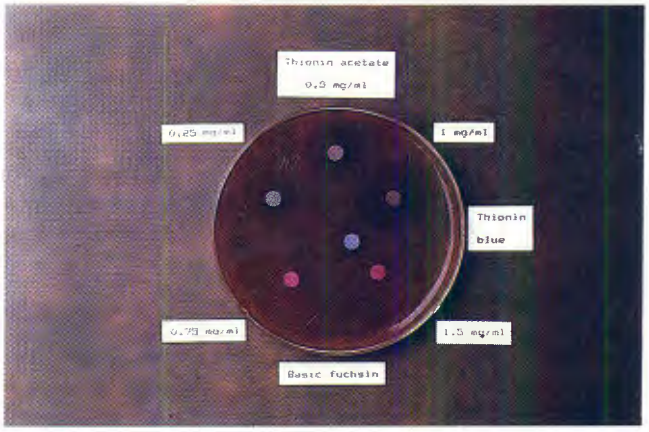

FIG. 2. *Brucella abortus* biotype 1 inhibited by thionin acetate (3 concentrations) discs on BTA

MATERIALS AND METHODS

Media

All *Brucella* cultures were grown on tryptose agar[1] with 10 % sterile citrated horse blood (BTA). The medium was poured into standard 90 mm diameter

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[1] Difco Laboratories, Detroit, Michigan, USA

TABLE 1 Growth of various *Brucella* strains in the presence of thionin acetate (Thio. acet.), basic fuchsin (Bas. fuc.) and thionin blue (T.b.) sensitivity discs at different concentrations expressed in mg/mℓ of solutions used

Brucella species	Air + 10 % CO_2						Air					
	Thio. acet.			Bas. fuc.		T.b.	Thio.acet.			Bas. fuc.		T.b.
	0,25	0,5	1	0,75	1,5	0,5	0,25	0,5	1	0,75	1,5	0,5
B. abortus bio. 1	–	–	–	+	+	+	NG	NG	NG	NG	NG	NG
B. abortus bio. 2	–	–	–	–	–	–	NG	NG	NG	NG	NG	NG
B. abortus S19	–	–	–	+	+	–	–	–	–	+	+	–
B. melitensis bio. 1	+	+	+	+	+	+	+	+	–	+	+	+
B. melitensis Rev. 1	+	+	–	+	+	+	+	–	–	+	–	+
B. ovis	+	+	+	–	–	+	NG	NG	NG	NG	NG	NG

\+ = positive growth (resistant)
– = negative growth (sensitive)
NG = no growth on plate (in air)

Petri dishes to give a depth of 3 mm once it solidified. Four plates were used for each *Brucella* strain.

Discs

Strips of grade 140 g/m² filter paper[2] were soaked in sterile solutions of thionin acetate[3] (0,25, 0,5 and 1 mg/mℓ), basic fuchsin[4] (0,75 and 1,5 mg/mℓ) and thionin blue[5] (0,5 mg/mℓ) for approximately 2 min and dried at 37 °C. Discs with a 5,5 mm diameter were cut out of the strips, placed in glass bottles and sterilized by autoclaving for 15 min at 121 °C. The discs were stored at 4 °C and a representative sample was incubated at 37 °C for 48 h on BTA to check for contaminants.

Strains

Reference strains NCTC 10093 (*B. abortus* biotype 1), NCTC 10501 (*B. abortus* biotype 2), NCTC 08038 (*B. abortus* strain 19), NCTC 10094 (*B. melitensis* biotype 1) and NCTC 10512 (*B. ovis*) were used, as well as *B. melitensis* Rev. 1 from the National Institute of Agronomical Research (INRA), Nouzilly, France.

Culture techniques

Forty-eight hour cultures of each strain were harvested from BTA plates and suspended in phosphate-buffered saline (PBS; pH 7,2) to a density of approximately 5×10^9 organisms per millilitre. One hundred µℓ of the suspension was pipetted onto each of 4 BTA plates, evenly spread over the whole surface of the plates with a glass spreader and allowed to dry. Discs of the various concentrations of thionin acetate, basic fuchsin and thionin blue were equidistantly placed onto each BTA plate (Fig. 1 & 2). All cultures were incubated at 37 °C, 2 plates of each strain in air and 2 plates in an atmosphere containing 10 % CO_2 (Corbel *et al.*, 1983). Cultures were examined after 48 and 96 h. Each strain was tested on 3 separate occasions.

Interpretation

A strain was regarded as sensitive to a particular dye or concentration when a clear zone of inhibition of 3 mm or more was seen around the relevant disc (Cruickshank, Duguid, Marmion & Swain, 1975; Pefanis, Venter & Herr, 1989) (Fig. 1 & 2).

Results and Discussion

The results of this investigation are summarized in Table 1. All *Brucella* strains reacted to the 3 dyes as described by Alton *et al.* (1975), Corbel *et al.* (1983), Corbel & Brinley-Morgan (1984) and Joint FAO/WHO (1986). The accuracy and consistency of these results seem to indicate that the use of discs is a reliable method for the dye sensitivity testing of *Brucella* strains. The 6 discs can easily be placed on a single BTA plate, therefore replacing the 6 dye plates which were previously used. This entails a saving in both media and dyes, as well as an economy of labour and space. The use of discs also provides for an easier and more accurate interpretation of results. This emanates from the easier standardization of materials and from the reduced subjectivity of interpretation. However, it must be born in mind that the inhibitory activity of each dye varies with its source and batch, and also with the culture medium used (Corbel *et al.*, 1983; Alton *et al.*, 1988). In this investigation, BTA was chosen in place of the commonly used SDA, as it provides a darker background, thus allowing for better visualization of the zones of inhibition.

Even though dye sensitivity testing is not by itself sufficient for the identification of all *Brucella* species, it is an essential part of the procedure. The reliability of the methods used is thus of great importance to instil confidence in the results obtained.

[2] Penpoint Stationers, P.O. Box 1457, Pretoria 0001
[3] Batch No. 9874570F, BDH Chemicals Ltd, Poole, England
[4] Batch No. 6977110K, BDH Chemicals Ltd, Poole, England
[5] Batch No. 287107, BDH Chemicals Ltd, Poole, England

References

ALTON, G. G., JONES, LOIS M. & PIETZ, D. E., 1975. Laboratory techniques in brucellosis, 2nd ed. Geneva: WHO.

ALTON, G. G., JONES, LOIS M., ANGUS, R. D. & VERGER, J. M., 1988. Techniques for the brucellosis laboratory. Paris: INRA.

CORBEL, M. J., GILL, K. P. W. & THOMAS, E. L., revised by CORBEL, M. J. & HENDRY, D. Mc. L. F. D., 1983. Methods for the identification of *Brucella*. Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England.

CORBEL, M. J. & BRINLEY-MORGAN, W. J., 1984. Genus *Brucella* Meyer and Shaw 1920. *In:* KRIEG, N. R. (ed.). Bergey's manual of systematic bacteriology, Vol. 1, 377–387. Baltimore, London: Williams & Wilkins.

CRUICKSHANK, R., DUGUID, J. P., MARMION, B. P. & SWAIN, R. H. A., 1975. Medical microbiology, Vol. 2, 12th ed.,

200–201. Edinburgh, London, New York: Churchill Livingstone.

FENSTERBANK, R., 1986. Brucellosis in cattle, sheep and goats: diagnosis, control and vaccination. *Revue scientifique et technique de l'Office International des Épizooties,* 5, 605–618.

JOINT FAO/WHO EXPERT COMMITTEE ON BRUCELLOSIS, 1986. World Health Organization Technical Report Series 740. Geneva: WHO.

PEFANIS, S. M., VENTER, CATHARINA G. & HERR, S., 1989. The use of cephalothin and triphenyltetrazolium chloride impregnated filter paper strips in the identification of *Campylobacter* species. *Onderstepoort Journal of Veterinary Research,* 56, 143–144.