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# THE EFFICACY OF BACTERIOLOGICAL PROCEDURES FOR THE ISOLATION OF *BRUCELLA ABORTUS* FROM ABATTOIR MATERIAL

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ABSTRACT

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A process of emulsifying and centrifuging abattoir specimens before plating out is described. *Brucella abortus* was isolated more successfully by this process than by conventional methods, especially in low grade infections. The 5 different media used were equally effective in our attempts at isolation, but growth did not necessarily occur on all 5 plates. In dairy cows, specimens from supramammary lymph nodes, udder and iliac lymph nodes accounted for a high percentage of positive isolations.

*Résumé*

*L'EFFICACITÉ DE PROCÉDÉS BACTERIOLOGIQUES POUR L'ISOLEMENT DE* BRUCELLA ABORTUS *A PARTIR DE MATÉRIEL D'ABATTOIR*

*Un procédé de broyage et de centrifugation d'echantillons d'abattoir avant l'encemencement de plaques de culture est décrit.* Brucella abortus *a été isolé avec plus de succès par ce procédé que par des méthodes conventionnelles, spécialement dans les infactions à faible degré. Les 5 milieux de culture utilisés furent tous également efficients dans nos tentatives d'isolement, mais la croissance ne survint pas nécessairement sur toutes les 5 milieux de culture. Chez les vaches laitières, les échantillons des ganglions lymphatiques supra-mammaires et iliaque et du pis, présentèrent un pourcentage élevé d'isolements positifs.*

## INTRODUCTION

Farrell (1974) described a selective medium for use in the isolation of *Brucella* from contaminated milk samples. This medium, to which an extra 5% horse serum was added to enhance growth, was used with 4 other media to determine whether any of the 5 gave consistently better results in our attempts at isolating the organism. A method of concentrating the organisms by centrifugation was also investigated to test whether an improvement in efficacy could be achieved.

Manthei & Carter (1950) and Stableforth & Galloway (1959) reported that the supramammary lymph nodes and udder were the best sites for isolating *Brucella,* but they did not indicate whether these 2 sites alone would account for 100% of the infected animals. Both Manthei & Carter (1950) and Alton, Jones & Pietz (1975) suggested that a series of abattoir specimens be used in attempts at isolation. As the udder and supramammary lymph nodes are removed from the carcass early in the slaughter process and can be handled without interfering with the slaughterline, we investigated the percentage of positive isolates which resulted from these organs alone.

## MATERIALS AND METHODS

### *Experimental animals*

The 10 animals used in this study were Friesian-type grade dairy cows originating from a small dairy herd. They had all shown some serological reaction to one or more Brucellosis tests as reported by Herr & Roux (1980).

### *Isolation procedures*

#### *Specimens*

Specimens were taken of the spleen, retropharyngeal lymph node, pooled left and right iliac lymph nodes, lumbar lymph nodes, pooled left and right supramammary lymph nodes, uterine wall and mammary tissue from the 4 quarters of each carcass and, in the case of pregnant animals, also from placenta, foetal lung, spleen and stomach content. A sterile scalpel was used to expose the tissue and a 2nd scalpel and a sterile rat-tooth forceps were used for excising the required specimen from each organ. Specimens were taken within 20 min of slaughter, placed in sterile, wide-mouthed, screw-capped bottles, kept at ambient temperature and processed within 4 hours.

#### *Processing of specimens*

A bacteriological needle was used to plate out material taken from a freshly cut surface of each organ. In an attempt to concentrate the organisms, the specimens in approximately 15 g quantities were first emulsified with 15 mℓ phosphate buffered saline (PBS)[1] in a Colworth[2] Stomacher 80 for about 30 seconds, and then made up to 50 mℓ with additional PBS. This suspension was centrifuged at 800 g for 10 min, the supernatant decanted and recentrifuged at 2 000 g for 20 min and the sediment then reconstituted with 0,2 mℓ PBS and plated out. The reconstituted sediments of both the lymph node and udder material from each animal were then separately pooled and both pools were once again plated out.

#### *Biological examination*

After the organisms had been concentrated by the method described above, the pooled lymph node and udder specimens as well as the spleen and uterine/placenta material from each animal was made up to 2 mℓ with PBS and 1 mℓ of each specimen injected intramuscularly into 2 guinea-pigs. One guinea-pig was sacrificed at 3 weeks and the other at 6 weeks post-inoculation. Blood collected in Alsever's solution and the spleen material were plated out on each occasion. Serum was taken at the same time for serological tests (Herr & Roux, 1980).

#### *Culture media and incubation methods*

Five different media were used throughout the study and each specimen was plated out on all 5 media.

*Medium* 1.—Serum dextrose agar (Davis[3]) plus antibiotics as described by Farrell (1974) and containing a total concentration of 10% horse serum.

*Medium* 2.—Tryptose agar (Biolab[4]) plus 10% horse blood.

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[1] Saline 0,15 M containing 0,01 M phosphate buffer pH 7,2
[2] A. J. Seward, UAC House, Blackfriars Rd., London SE1 9UG.
[3] Davis Gelatin (N.Z.) Ltd., Christchurch, New Zealand
[4] Biolab, 4 Bernard St., Colbyn, Pretoria, R S A

TABLE 1 Approximate *B. abortus* colony counts on primary isolation by direct plating of specimens

| Cow No. | Organ material | Specimen processing method | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Direct plating from cut surface | | | | | Concentration before plating | | | | | Concentrates pooled before plating | | | | | |
| | | Medium* | | | | | Medium* | | | | | Organs | Medium* | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | | 1 | 2 | 3 | 4 | 5 |
| 1216 | Spleen | — | — | — | — | — | 1 | — | 2 | — | — | Lymph nodes | — | — | — | — | — |
| | Lymph nodes: | | | | | | | | | | | Udder | 100 | 20 | 80 | 70 | 100 |
| | Retropharyngeal | — | — | — | — | — | 1 | — | — | — | — | | | | | | |
| | Iliac | — | 1 | 1 | — | 6 | 20 | 25 | 30 | 20 | 20 | | | | | | |
| | Lumbar | — | 5 | — | 10 | — | 20 | 20 | 30 | 30 | 50 | | | | | | |
| | Supramammary | 50 | 50 | 20 | 100 | 80 | 100 | 100 | 50 | 50 | 50 | | | | | | |
| | Placenta/uterus | 4 | 2 | 2 | 1 | 1 | 1 | — | 2 | — | — | | | | | | |
| | Udder quarters: | | | | | | | | | | | | | | | | |
| | 1 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 80 | 100 | | | | | | |
| | 2 | 30 | 80 | 30 | 30 | 50 | 80 | 100 | 100 | 50 | 100 | | | | | | |
| | 3 | 12 | 20 | 20 | 20 | 10 | 80 | 60 | 40 | 80 | 70 | | | | | | |
| | 4 | 100 | 100 | 100 | 100 | 100 | 50 | 100 | 100 | 70 | 100 | | | | | | |
| 1269 | Spleen | — | — | — | — | — | — | — | — | — | — | Lymph nodes | 9 | — | 10 | 1 | 2 |
| | Lymph nodes: | | | | | | | | | | | Udder | — | — | — | — | — |
| | Retropharyngeal | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | Iliac | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | Lumbar | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | Supramammary | — | 1 | — | 3 | — | 23 | 40 | 30 | 32 | 30 | | | | | | |
| | Placenta/uterus | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | Udder quarters: | | | | | | | | | | | | | | | | |
| | 1 | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | 2 | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | 3 | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | 4 | — | — | — | — | — | — | — | — | — | — | | | | | | |
| 2026 | Spleen | — | — | — | 1 | — | — | — | — | — | — | Lymph nodes | 80 | 100 | 100 | 100 | 100 |
| | Lymph nodes: | | | | | | | | | | | Udder | 80 | 100 | 100 | 200 | 100 |
| | Retropharyngeal | 20 | 60 | 50 | 50 | 100 | 40 | 50 | 80 | 100 | 100 | | | | | | |
| | Iliac | 4 | 1 | — | 2 | 1 | 5 | 7 | 4 | 7 | 20 | | | | | | |
| | Lumbar | 1 | 2 | 1 | 1 | — | 3 | 4 | 4 | 1 | 6 | | | | | | |
| | Supramammary | 4 | 12 | 4 | 8 | 8 | 100 | 100 | 100 | 100 | 100 | | | | | | |
| | Placenta/uterus | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | | | | | | |
| | Udder quarters: | | | | | | | | | | | | | | | | |
| | 1 | — | — | — | — | — | 4 | — | 2 | 2 | 4 | | | | | | |
| | 2 | 10 | 3 | 5 | 6 | 10 | 15 | 30 | 20 | 10 | 10 | | | | | | |
| | 3 | 70 | 70 | 50 | 70 | 80 | 80 | 100 | 100 | 100 | 100 | | | | | | |
| | 4 | — | — | — | — | — | — | — | — | 2 | — | | | | | | |

TABLE 1 Approximate *B. abortus* colony counts on primary isolation by direct plating of specimens (*Continued*)

| Cow No. | Organ material | Specimen processing method | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Direct plating from cut surface | | | | | Concentration before plating | | | | | Concentrates pooled before plating | | | | | |
| | | Medium* | | | | | Medium* | | | | | Organs | Medium* | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | | 1 | 2 | 3 | 4 | 5 |
| 9341 | Spleen | — | — | — | — | — | — | 1 | — | — | — | Lymph nodes | — | — | 2 | — | — |
| | Lymph nodes: | | | | | | | | | | | Udder | — | 3 | 2 | 2 | 1 |
| | Retropharyngeal | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | Iliac | 1 | — | — | — | — | 2 | 1 | — | — | 1 | | | | | | |
| | Lumbar | 1 | — | — | 1 | 1 | — | 1 | 1 | — | — | | | | | | |
| | Supramammary | — | — | — | — | 5 | — | — | — | — | — | | | | | | |
| | Placenta/uterus | — | — | — | — | — | 3 | 10 | 5 | 4 | 10 | | | | | | |
| | Udder quarters: | | | | | | | | | | | | | | | | |
| | 1 | — | 1 | 6 | — | — | — | 1 | — | 1 | 3 | | | | | | |
| | 2 | 20 | 40 | 30 | 15 | 20 | 3 | 4 | — | 2 | 2 | | | | | | |
| | 3 | 20 | 20 | 20 | 8 | 15 | — | 3 | 3 | 1 | 2 | | | | | | |
| | 4 | — | — | — | — | — | — | — | — | — | — | | | | | | |
| 9542 | Spleen | — | — | — | — | — | — | — | — | — | — | Lymph nodes | — | — | — | — | — |
| | Lymph nodes: | | | | | | | | | | | Udder | — | — | — | — | — |
| | Retropharyngeal | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | Iliac | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | Lumbar | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | Supramammary | — | 2 | — | 2 | — | 2 | 5 | — | 1 | 5 | | | | | | |
| | Placenta/uterus | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | Udder quarters: | | | | | | | | | | | | | | | | |
| | 1 | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | 2 | 4 | 1 | — | 1 | 1 | 20 | 20 | 8 | 17 | 15 | | | | | | |
| | 3 | — | — | — | — | — | — | — | — | — | — | | | | | | |
| | 4 | — | — | — | — | — | — | — | — | — | — | | | | | | |

*MEDIA 1 Serum dextrose agar plus antibiotics
2 Tryptose agar plus 10% horse blood
3 Tryptose agar plus 10% horse blood plus antibiotics and dye
4 Brain/heart infusion agar plus 10% horse blood
5 Brucella agar plus 5% horse blood

*Medium* 3.—Tryptose agar (Biolab[4]) plus 10% horse blood plus antibiotics and dye as in Albimi agar (Cruikshank, Duguid, Marmion & Swain, 1975).

*Medium* 4.—Brain/heart infusion (Difco[5]) agar (Davis[3]) plus 10% horse blood.

*Medium* 5.—Brucella agar (Difco[5]) plus 5% horse blood.

All plates were incubated at 37 °C with 10% $CO_2$ added (Forma Scientific $CO_2$ Incubator) and examined both macro- and microscopically for the presence of *B. abortus* colonies after 48, 96 and 144 hours.

*Typing of isolates*

The isolates were typed according to World Health Organisation standards (Alton *et al.*, 1975), using the criteria of aerobic growth, growth on 5 tryptose agars (Biolab[4]) with 1/50 000 or 1/100 000 basic fuchsin or 1/25 000, 1/50 000 or 1/100 000 thionin and growth on 3 *Brucella* agars (Difco[5]) with penicillin 5 000 IU/ℓ, erythritol 1 g/ℓ or streptomycin 2,5 mg/ℓ. The fluorescent antibody test, using *B. abortus* and *B. ovis* conjugates prepared as described by Kito, Ogimoto & Suto (1966), monospecific serum agglutination using *B. abortus* and *B. melitensis* antigens (Wellcome[6]), the acriflavine test 1 g/ℓ and routine test dilution of Tbilisi *Brucella* phage (C.V.L.[7]) were also employed.

## Results

All the isolates were *Brucella abortus* biotype 1. The approximate colony counts on the various plates and the different methods of processing specimens are set out in Table 1. No isolations were made from 5 of the cows and their results are not shown in the tables. The degree of contamination was negligible on all the plates, but the serum dextrose agar with antibiotics, as recommended by Farrell (1974), rarely showed any contamination at all.

*Effect of concentration*

The effort at concentration was the most rewarding in the cases where a low grade infection existed, and this is confirmed by the dramatic increase in colony counts following the above process as well as by the fact that growth occurred on more of the plates (e.g. 1216 iliac lymph node in Table 1). With a few exceptions, this trend was noticeable throughout the study. Where a high grade infection existed, however, this pattern was hardly noticeable. The pooling of the concentrated specimens had the opposite effect and this is thought to be due to a dilution factor.

*Effect of different media*

No single medium gave consistently better results than the others.

*Biological isolation*

Cultural results following the inoculation of the 2 guinea-pigs are presented in Table 2. The results show that the method is marginally more sensitive than direct bacteriological isolation and that this sensitivity increases with the longer incubation period (42 days). Isolations from guinea-pig blood collected in Alsever's solution were very poor compared with those from guinea-pig spleen specimens and with direct bacteriological isolation. Once again no specific medium gave markedly better results than any other.

*Serological diagnosis in guinea-pigs*

Serological results following on the inoculation of guinea-pigs were largely in agreement with the isolation results and are reported more fully in a second paper (Herr & Roux, 1980). A single case from which no isolate could be made was serologically positive 42 days after the guinea-pigs had been inoculated.

## Discussion

*Selection of abattoir specimens*

Although the number of animals included in this study is too small to base any statistical analysis on, the trend with regard to organs having the best potential for isolating *B. abortus* (Table 3) is in agreement with the findings of Stableforth & Galloway (1959) and Manthei and Carter (1950). It would appear that, at least in dairy cattle, *B. abortus* tends to locate in the udder and supramammary lymph nodes and that a high percentage (100% in this small trial) of infected animals will yield positive cultures from these 2 sites. However, it must be stressed that individual quarters should be cultured, as pooling tends to have a diluting effect where one or more quarters are negative (Table 1). It is suggested therefore, that the udder tissue (from separate specimens taken from each quarter), and the supramammary lymph nodes (pooled) plus the iliac lymph nodes (pooled), a total of 6 specimens from each carcass, are sufficient to give a very accurate picture of the presence of *Brucella* organisms in dairy cattle. The practical advantage of these specimens lies in the fact that the slaughter-line need only be slightly interrupted for taking a sample of the iliac lymph nodes, while the other specimens may be collected in an orderly manner well away from slaughter activities. From Table 3 it can be seen that attempts at isolation from retropharyngeal lymphnodes and spleen are seldom successful even in known positive cases and that their inclusion in any series is therefore of doubtful value. Whether this is equally applicable to infection in beef cattle of the *Bos taurus* and *B. indicus* types and in young stock calls for further investigation.

*Biological isolation*

The marginal increase in sensitivity seen in biological isolation makes this process hardly worth the time and work involved except that 1 case proved serologically positive after the guinea-pig inoculation. The methods currently in use for culturing from guinea-pig blood appear to be totally inadequate.

*Concentration process and efficacy of media*

The process of concentrating the organisms before plating gives excellent results, especially in cases with a low grade infection, and it seems well worth while adopting it as a routine measure. The use of 5 different media per specimen is to be recommended, as low grade infections frequently do not show up on all the plates. This may, however, be equally true if 5 plates of the same medium were used. The inclusion

[3] Davis Gelatin (N.Z.) Ltd., Christchurch, New Zealand
[4] Biolab, 4 Bernard St., Colbyn, Pretoria, R S A
[5] Difco Lab., Detroit, Michigan, U.S.A.
[6] Wellcome Reagents Ltd., Beckenham, England, BR3 3BS
[7] Central Veterinary Laboratory, New Haw, Weybridge, England

TABLE 2 Cultural results following guinea-pig inoculation

| Cow No. | Organ material | Specimens collected 21 days after inoculation | | | | | | | | | | Specimens collected 42 days after inoculation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Spleen | | | | | Blood | | | | | Spleen | | | | | Blood | | | | |
| | | Medium* | | | | | Medium* | | | | | Medium* | | | | | Medium* | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| 1216 | Lymph node pool | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | Udder pool | + | + | + | + | + | — | — | — | — | — | + | + | — | + | + | — | — | — | — | — |
| | Spleen | + | + | + | + | + | — | — | — | — | — | + | + | — | + | + | — | — | — | — | — |
| | Uterus/placenta | + | + | + | + | + | + | — | — | — | — | + | + | — | + | + | — | — | — | — | — |
| 1269 | Lymph node pool | + | + | + | + | + | — | — | — | — | + | + | + | + | + | + | — | — | — | — | — |
| | Udder pool | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | Spleen | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | Uterus/placenta | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 2026 | Lymph node pool | + | + | + | + | + | — | — | — | — | — | + | + | + | + | + | — | — | — | — | — |
| | Udder pool | + | + | + | + | + | — | — | — | — | — | + | + | — | + | + | — | — | — | — | — |
| | Spleen | — | — | — | — | — | — | — | — | — | — | + | + | — | + | + | — | — | — | — | — |
| | Uterus/placenta | + | + | + | + | + | — | — | — | — | — | + | + | — | + | + | — | — | — | — | — |
| 9341 | Lymph node pool | — | — | — | — | — | — | — | — | — | — | + | + | — | + | + | — | — | — | — | — |
| | Udder pool | + | + | + | + | + | — | — | — | — | — | + | — | — | + | + | — | — | — | — | — |
| | Spleen | — | — | — | — | — | — | — | — | — | — | + | + | + | + | + | — | — | — | — | — |
| | Uterus/placenta | + | + | + | + | + | — | — | — | — | — | + | + | + | + | + | — | — | — | — | — |
| 9542 | Lymph node pool | + | + | + | + | + | — | — | — | — | — | + | + | + | + | + | — | — | — | — | — |
| | Udder pool | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | Spleen | + | + | + | + | + | — | — | — | — | — | + | + | + | + | + | — | — | — | — | — |
| | Uterus/placenta | + | + | + | + | + | — | — | — | — | — | + | + | + | + | + | — | — | — | — | — |

*MEDIA 1 Serum dextrose agar plus antibiotics
2 Tryptose agar plus 10% horse blood
3 Tryptose agar plus 10% horse blood plus antibiotics and dye
4 Brain/heart infusion agar plus 10% horse blood
5 Brucella agar plus 5% horse blood

— = No colony growth + = Presence of *B. abortus colonies*

TABLE 3 Total number of positive cows according to isolates from various organs and processing systems

| Organ | Direct plating | Concentration before plating | Pooled concentrates | |
|---|---|---|---|---|
| | | | *Organ* | |
| Spleen | 1 | 2 | Lymph nodes | 3 |
| Lymph nodes (lnn): | | | Udder | 3 |
| Retropharyngeal | 1 | 2 | | |
| Iliac | 3 | 3 | | |
| Lumbar | 3 | 3 | | |
| Supramammary | 4 | 4 | | |
| Placenta | 2 | 3 | | |
| Udder—all 4 quarters | 4 | 4 | | |
| Udder plus supramammary lnn | 5 | 5 | | |
| Supramammary plus iliac lnn | 5 | 5 | | |
| Udder plus iliac lnn | 4 | 4 | | |

of a variety of media has the advantage that those with added antibiotics will take care of possibly contaminated sources, while better and faster colony growth is often seen on the media without antibiotics. Contrary to the findings of Farrell (1974), no single medium in this study could be classed as giving consistently better results than any other. Farrell (1974), however, attributed the better results with his medium to the suppression of contaminants, which was not really a problem in this series. Growth on the different media appears to be a function of the degree of infection rather than of the suitability of the media. Wherever the lowest colony count exceeded 10 colonies per plate, growth was seen on all the media.

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