

QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES

DIVISION OF ANIMAL INDUSTRY BULLETIN No. 168

**COMPARISON OF ISOLATION METHODS OF
ERYSIPELOTHRIX RHUSIOPATHIAE FROM
ARTHRITIC PIG JOINTS**

by P. J. BLACKALL, B.Sc.; and P. M. SUMMERS, B.V.S., M.Sc.

SUMMARY

A practical routine isolation technique is established for *Erysipelothrix rhusiopathiae* from arthritic pig joints. A direct culture is made onto selective erysipelo-thrix agar and non-selective blood agar plates. The joint fluid and synovial membrane is stored in an enrichment broth at 4°C for 4 weeks and then cultured onto erysipelo-thrix agar and blood agar plates.

I. INTRODUCTION

Arthritis is a significant cause of condemnations of pigs at slaughter in Australia (Seddon 1965; Cross, Penny and Claxton 1971; Bond 1976). Although *Erysipelothrix rhusiopathiae* is known to be one of the causes of arthritis in pigs (Shuman and Wood 1970), isolation rates of the organism from arthritic pig joints are often disappointingly low (Ward 1922; Craig 1926; Collins and Goldie 1940; Ajmal 1969).

Previous work has shown that the isolation rate of *E. rhusiopathiae* from arthritic pig joints was increased following the storage of joints at 4°C for varying periods of time up to 10 weeks (P. J. Ketterer, personal communication 1974). However, because of the impracticability of prolonged storage of joints, further studies were undertaken to establish a more practical isolation technique for *E. rhusiopathiae*.

II. MATERIALS AND METHODS

A total of 129 joints was collected at the abattoirs from pigs observed to have arthritis at meat inspection. The majority of the joints collected were stifle joints. After examination of the joints for pathological features, portions of the joint fluid and synovial membrane were collected aseptically and inoculated into 10 ml of a liver enriched peptone broth.

Three methods of isolation of *E. rhusiopathiae* were used.

Method A (Direct culture)

Using a standard 4-mm bacteriological loop, the inoculated enrichment medium was plated directly onto blood agar and erysipelo-thrix agar (Packers Medium, Packer 1943) plates. Both plates were incubated aerobically at 37°C for 4 days. Suspect colonies were identified according to the criteria of Wood (1970).

Method B (Enrichment)

A 5-ml sample of the inoculated enrichment medium was pipetted into a sterile 25-ml bottle. This volume was incubated at 37°C for 2 days. At the end of this period, the medium was plated onto blood agar and erysipelothrux agar plates (again using a 4-mm bacteriological loop). The plates were incubated aerobically at 37°C for 4 days and suspect colonies identified as before.

Method C (Cold temperature incubation)

The remaining volume of inoculated enrichment medium was stored at 4°C. After 4, 8, 12 and 16 weeks, it was sub-cultured using a 4-mm bacteriological loop onto blood agar and erysipelothrux agar plates. The plates were incubated aerobically at 37°C for 4 days and suspect colonies identified as before.

III. RESULTS

The number of isolates of *E. rhusiopathiae* obtained using the three isolation methods is given in table 1. *E. rhusiopathiae* was isolated from 28 (21.7%) joints.

The results were analysed statistically using the Chi square test to the 95% confidence level. There was no significant difference between the isolation rate obtained by direct culturing (Method A) and that obtained by using 2-day enrichment (Method B). However, there was a significant difference between the isolation rate obtained after 4 weeks at 4°C (Method C) and that obtained by either direct culture (Method A) or the 2-day enrichment (Method B). Further cold storage beyond 4 weeks did not significantly increase the isolation rate.

There was no significant difference between the isolations obtained on blood agar and those obtained on erysipelothrux agar at direct culturing and after 2-days enrichment. However, after 4-weeks cold storage there was a significant difference, more isolates being obtained on the erysipelothrux agar.

Two isolates of *E. rhusiopathiae* were obtained by means of the 2-day enrichment and by no other means. While no single isolation method gave all 28 isolates of *E. rhusiopathiae*, the combinations of direct culturing and 4-weeks cold storage and direct culturing and 8-weeks cold storage both gave 26 isolates of *E. rhusiopathiae*.

TABLE 1
COMPARISON OF NUMBERS OF ISOLATES OF *E. rhusiopathiae* OBTAINED USING ISOLATION METHODS A, B, C

Isolation Method	Blood Agar	Erysipelothrux Agar	Total Isolates
A — Direct	16	17	17
B — 2-Day Enrichment	15	18	18
{ 4-weeks Storage	15	24	24
{ 8-weeks Storage	19	26	26
{ 12-weeks Storage	24	22	25
{ 16-weeks Storage	19	18	23

IV. DISCUSSION

The use of cold storage to increase the isolation rate of *E. rhusiopathiae* is widely accepted (Wood 1970). The results obtained in this work have confirmed this fact and in addition indicated that only a limited cold-storage period is necessary to achieve the most effective isolation rate. Beyond 4 weeks no further significant increase was achieved indicating that prolonged cold storage did not result in a continual rise in the isolation rate of *E. rhusiopathiae*.

The use of an enrichment technique for the isolation of *E. rhusiopathiae* appeals as a rapid method and has been recommended by several workers (Wood 1965; Timoney and Berman 1970; Bond 1976). However in this study the incubation of joint material in the liver-enriched peptone broth for 2 days did not achieve an increased isolation of *E. rhusiopathiae*. It should be noted that the enrichment medium differed from that of Wood (1965) by not containing antibiotics. However, the failure of the enrichment medium to significantly increase the isolation rate of *E. rhusiopathiae* could not be attributed to this inability to inhibit the growth of contaminants. This was established by the fact that the number of isolates obtained on the non-selective blood agar was not significantly different from that obtained on the selective erysipelothrix agar.

The use of a selective medium for the culturing of *E. rhusiopathiae* has long been recommended (Packer 1943). The results obtained in our studies showed that if direct culturing is used then contamination is at such a low level that the selective medium is not necessary. In contrast the selective medium does become necessary after cold storage as contamination increases.

V. ACKNOWLEDGEMENTS

The assistance of the meat inspectors who submitted joints is gratefully acknowledged. The preparation of the enriched peptone broth was based on information supplied by Dr M. E. Nairn, now at Murdoch University, Western Australia. The work was funded by grants from the Australian Pig Industry Research Committee.

REFERENCES

- AJMAL, M. (1969).—*Erysipelothrix rhusiopathiae* and spontaneous arthritis in pigs. *Res. vet. Sci.* 10: 579-582.
- BOND, M. P. (1976).—Polyarthritis of pigs in Western Australia: role of *E. rhusiopathiae*. *Aust. vet. J.* 52: 462-467.
- COLLINS, D. H. AND GOLDIE, W. (1940).—Observations on polyarthritis and on experimental erysipelothrix infection of swine. *J. Path. and Bact.* 50: 323-353.
- CRAIG, J. F. (1926).—Some observations of swine erysipelas. *Vet. Rec.* 6: 849-856.
- CROSS, G. M., PENNY, R. H. C. AND CLAXTON, P. D. (1971).—The abattoir incidence of polyarthritis in pigs in Australia. *Aust. vet. J.* 47: 126.
- PACKER, R. A. (1943).—The use of sodium azide (NaN_3) and crystal violet in a selective medium for streptococci and *E. rhusiopathiae*. *J. Bacteriol.* 46: 343-349.
- SEDDON, H. R. (1965).—Erysipelothrix infections. In 'Diseases of Domestic Animals in Australia'. Part 5 Vol. 1 pp 127-138 Department of Health, Commonwealth of Australia.

- SHUMAN, R. D. AND WOOD, R. L. (1970).—Swine erysipelas. In 'Diseases of Swine' pp 508-562. Iowa State University Press; Ames, Iowa.
- TIMONEY, J. F. AND BERMAN, D. T. (1970).—Erysipelothrix arthritis in swine. Bacteriologic and immunopathogenic aspects. *Am. J. vet. Res.* 31: 1411-1421.
- WARD, A. R. (1922).—The aetiology of polyarthritis in swine. *J. Am. vet. Med. Assoc.* 61: 155-161.
- WOOD, R. L. (1965).—A selective liquid medium utilizing antibiotics for isolation of *E. rhusiopathiae*. *Am. J. Vet. Res.* 26: 1303-1308.
- WOOD, R. L. (1970).—Erysipelothrix. In 'Manual of Clinical Microbiology' pp 101-105. American Society for Microbiology; Bethesda.

(Received for publication 25 July 1977).

The authors are officers of Pathology Branch, Queensland Department of Primary Industries and are stationed at the Animal Research Institute, Yeerongpilly, Queensland, 4105.