

From the Department of Food Hygiene and the Department of Microbiology and Immunology, Veterinary College of Norway, Oslo.

OBSERVATIONS ON THE UTILIZATION OF A SELECTIVE MEDIUM FOR THE ISOLATION OF ERYSIPELOTHRIX RHUSIOPATHIAE

By

Anna Marie Bratberg

BRATBERG, ANNA MARIE: *Observations on the utilization of a selective medium for the isolation of Erysipelothrix rhusiopathiae.* Acta vet. scand. 1981, 22, 55—59. — A medium containing 3 antibiotics: neomycin, kanamycin and vancomycin for the selective culture of *Erysipelothrix rhusiopathiae* was studied during various culture tests. It was found that 6 out of 35 previously identified strains of *E. rhusiopathiae* (17 %) showed no growth or variable growth on the medium. It appeared that the strains with the highest sensitivity for kanamycin were inhibited by the medium. A number of other laboratory strains of microorganisms did not grow on the medium, indicating a good selective effect. Several resistant microorganisms were, however, found in field material. A small number of isolation attempts using tonsils of swine and sheep revealed a very low occurrence (4 % in swine) of *E. rhusiopathiae*.

Erysipelothrix rhusiopathiae; selective medium; isolation.

A liquid medium for the selective culture of *Erysipelothrix rhusiopathiae* was described by *Wood* in 1965. It consisted of a base medium enriched with horse serum and 3 different antibiotics, which were added to obtain selective effect. The medium was developed mainly for the isolation of *E. rhusiopathiae* from pig faeces. This selective medium has been used by *Wood & Packer* (1972), who studied the occurrence of *E. rhusiopathiae* in soil and manure from swine pens, and by *Stephenson & Beriman* (1978) for isolation of *E. rhusiopathiae* from swine tonsils. *Nielsen* (1969) used this selective medium in a growth agglutination test to demonstrate chronic infections caused by *E. rhusiopathiae*. When the medium was introduced in our laboratory, it

was found that a reference strain of *E. rhusiopathiae* did not grow on it. Following this experience, cultivation attempts were performed using previously identified strains of *E. rhusiopathiae* and a number of other microorganisms, representing various groups of microorganisms suspected to interfere with the selective effect. The sensitivity to the antibiotics used in the medium was tested, and the medium was used for the isolation of *E. rhusiopathiae* from field material.

The aim of this paper is to direct attention to questionable factors in the isolation method as revealed during these experiments.

MATERIALS AND METHODS

Thirty-five strains of *E. rhusiopathiae* were tested. Seven isolates originated from various clinical forms of the disease in swine, 6 isolates were from sheep, 1 from a mouse, 4 from turkeys, 2 from poultry, 1 from an eagle and 3 from fish. The remaining 11 strains were of unknown origin. Some of the strains had been typed according to previously used systems designated by letters (for reference see review by *Kucsera* 1973). Among the swine strains types A₁, A₂, B₁, B₂ and H occurred. The fish strains belonged to types D, E and G. Among the strains of unknown origin were types A, B, C and N. Other microorganisms cultivated were: *Escherichia coli* (non-haemolytic), *E. coli* (haemolytic), *Salmonella typhimurium*, *Klebsiella*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Pasteurella multocida*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Lactobacillus* No. 2477, *Listeria monocytogenes*, *Corynebacterium pyogenes* and *Candida albicans*.

The field material used in isolation experiments consisted of tonsils collected post mortem from 19 pigs and 6 sheep, and tonsils from 31 apparently healthy pigs collected at an abattoir.

The liquid base medium was made from Bacto Infusion Broth (Difco B 344) to which 10 % horse serum was added, the pH adjusted to 7.8. A solid medium was made from the same ingredients by adding 1.5 % agar. The antibiotics added were: Neomycin-sulphate 50 µg/ml, kanamycin 400 µg/ml (Kantrex®, Bristol Lab., UK), vancomycin 25 µg/ml (Vancocin®, Eli Lilly & Co, Indianapolis, USA) as recommended by *Wood* (1965).

Sensitivity tests against neomycin, kanamycin and vancomycin were performed by the dish method using Neo-Sensitabs®

(Rosco, Denmark). The tests were carried out as described by the manufacturer. Blood agar was used as the test medium. The isolation attempts were carried out as described by *Stephenson & Berman* (1978), except that the subcultures were inoculated in parallel on solid selective medium and on blood agar. Colonies resembling *E. rhusiopathiae* were examined for morphological, cultural and biochemical properties as criteria for classification (*Selliger* 1974).

RESULTS

Three of the 35 strains studied did not grow on the selective medium. These were an isolate from lamb arthritis and 2 strains of unknown origin. Three other strains showed variable growth on the selective medium. These were an isolate from swine labelled type A₁, an isolate from lamb arthritis, and a strain of unknown origin labelled type A. The other 29 strains grew on the selective medium as described by *Wood* (1965). When grown on the solid selective medium, the colonies were "pin-point" after 24 h at 37°C. After 72 h the colonies were 1—1½ mm in diameter, and smooth with atwhite to bluish-white colour. All the strains of *E. rhusiopathiae* were inhibited by neomycin and vancomycin ranging in degree from "slightly sensitive" to "sensitive". Four strains showed kanamycin sensitivity zones corresponding to "slightly sensitive" or "fairly sensitive". Three of these were the strains that did not grow on the selective medium, while the fourth was the strain type A that showed variable growth. The other bacteria tested did not grow on the medium. *Candida albicans* grew in white opaque colonies, 1—1½ mm in diameter after 48 h at 37°C.

E. rhusiopathiae was isolated from 2 of the 19 pig tonsils and from 1 of the 6 sheep tonsils from pathological cases. The 2 positive porcine samples were both from piglets, which had died from haemorrhagic enteritis and pneumonia respectively. The positive sheep sample was from a 2 year old ewe in which thrombotic endocarditis caused by *E. rhusiopathiae* was diagnosed. All the tonsils from the apparently healthy swine were negative for *E. rhusiopathiae*. In the positive cases the bacteria were found both on the solid selective medium and on blood agar. In 20 % of the isolation cultures a coliform bacteria resistant to the 3 selective antibiotics was found. In a few cultures molds or yeasts were found.

DISCUSSION

Six of the 35 strains of *E. rhusiopathiae* studied (17 %) showed no growth or variable growth on the selective medium. Among these strains were isolates from pathological cases of swine and sheep. When establishing the content of antibiotics in the selective medium Wood (1965) used 8 strains of *E. rhusiopathiae* all isolated from swine: 6 strains were from septicaemic cases and 2 strains showed reduced virulence in swine. They represented serotypes A and B. The variation within the *E. rhusiopathiae* species seems, however, to extend beyond this test material.

The sensitivity tests showed a greater inhibition than expected for all 3 antibiotics. This can probably be attributed to the method having been standardized with faster growing bacteria. The diffusion of antibiotics from the tablets will extend before the slow growing bacteria have multiplied to form visible colonies. An inability to grow in the selective medium corresponds to some degree with a moderate sensitivity for kanamycin. As kanamycin and neomycin are known to have a partially synergistic effect, it might be that the combination of these 2 antibiotics at low concentrations affects the strains most sensitive to kanamycin. To ensure the detection of these strains in isolation tests using this selective medium, kanamycin could possibly be added in smaller concentrations or replaced by another antibiotic. The inhibitory effect on other microorganisms seems to be good when attempting cultivation with laboratory strains of various microorganisms. Field material, however, contained several microorganisms resistant to the antibiotics used in the medium. On the subcultures from the cases positive for *E. rhusiopathiae*, colonies of *E. rhusiopathiae* were found in large numbers despite the growth of unwanted microorganisms on both types of agar plates tested. *Stephenson & Berman* (1978) used another solid selective medium containing sodium azide and crystal violet for subcultivation. This might have excluded the contaminants. When attempting isolation from pig tonsils remarkably few positives (4 %) were found when compared with a rate of 98 % described by *Stephenson & Berman*. This could be due to a generally low incidence of the microorganism in Norway and a possible seasonal variation throughout the year. The tests were performed in January while the disease mainly occurs in the late summer. Furthermore the selective medium that was used might have excluded individual

strains. No references describing the occurrence of *E. rhusiopathiae* in sheep tonsils have been found. The isolation of the microorganism from the tonsils of a sheep that died from endocarditis caused by *E. rhusiopathiae* must be considered an exceptional case.

REFERENCES

- Kucsera, G.*: Proposal for standardization of the designations used for serotypes of *Erysipelothrix rhusiopathiae*. *Int. J. system. Bact.* 1973, 23, 184—188.
- Nielsen, N. C.*: The use of a selective medium in the growth-agglutination test for chronic *Erysipelothrix insidiosa* infections in swine. *Acta vet. scand.* 1969, 10, 127—136.
- Selliger, H. P. R.*: Genus *Erysipelothrix*. In Buchanan, R. E. & N. E. Gibbons, eds. *Bergey's Manual of Determinative Bacteriology*. 8th Ed. Williams & Wilkins Company, Baltimore 1974, p. 597.
- Stephenson, E. H. & D. T. Berman*: Isolation of *Erysipelothrix rhusiopathiae* from tonsils of apparently normal swine by two methods. *Amer. J. vet. Res.* 1978, 39, 187—188.
- Wood, R. L.*: A selective liquid medium utilizing antibiotics for isolation of *Erysipelothrix insidiosa*. *Amer. J. vet. Res.* 1965, 26, 1303—1308.
- Wood, R. L. & R. A. Packer*: Isolation of *Erysipelothrix rhusiopathiae* from soil and manure of swine-raising premises. *Amer. J. vet. Res.* 1972, 33, 1611—1620.

SAMMENDRAG

Erfaringer ved bruken av et selektivt medium til isolering av Erysipelothrix rhusiopathiae.

Et medium som inneholder tre antibiotika: neomycin, kanamycin og vancomycin til selektiv oppformering av *Erysipelothrix rhusiopathiae* ble undersøkt gjennom forskjellige dyrkningsforsøk. Det ble funnet at 6 av 35 på forhånd diagnostiserte *E. rhusiopathiae*-stammer (17 %) ikke vokste eller ikke ga konstant vekst i mediet. Det synes å være de stammer som hadde størst følsomhet for kanamycin som ble hemmet av mediet. Laboratoriestammer av andre mikroorganismer vokste ikke på mediet hvilket indikerte en god selektiv effekt. Ved oppformering fra biologisk materiale ble det imidlertid registrert en del resistente mikroorganismer. Resultater av et begrenset antall forsøk på isolering av *E. rhusiopathiae* fra tonsiller på gris og sau indikerte liten utbredelse (4 % fra gris) av mikroorganismen her i landet.

(Received October 17, 1980).

Reprints may be requested from: Anna Marie Bratberg, the Department of Food Hygiene, Veterinary College of Norway, P. O. Box 8146, Dep., Oslo 1, Norway.