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A Selective Medium for the Primary Isolation of *Haemophilus pertussis* and *Haemophilus paraptussis*

By D. E. NICHOLSON AND G. C. TURNER

Department of Bacteriology, School of Medicine, Leeds

SUMMARY: A description is given of a selective medium which supports the growth of *Haemophilus pertussis* and *Haemophilus paraptussis* and largely inhibits the growth of *Haemophilus influenzae*. The medium contains horse-meat extract, horse blood, salt agar, 2 μ g. 4:4'-diamidino-diphenylamine-dihydrochloride/ml. and 0.3 unit penicillin/ml. The medium is of value in the primary isolation of *H. pertussis* and *H. paraptussis*.

Dawson, Farnworth, McLeod & Nicholson (1951) reported that a medium containing beef extract and horse blood was able to support the growth of *Haemophilus pertussis* while considerably inhibiting the growth of *H. influenzae*. This inhibitory effect was shown to be associated with the horse blood, and we have since shown that an even greater inhibitory effect can be obtained by using horse-meat extract instead of beef extract. During the course of this work Lacey (1951) described a medium which was highly selective for *H. pertussis* containing 0.3 units penicillin/ml. and 12 μ g. 4:4'-diamidino-diphenylamine-HCl/ml. In view of Lacey's findings we investigated the possibility of incorporating 4:4'-diamidino-diphenylamine-HCl in our medium. It was shown that the growth of *H. influenzae* was completely suppressed by one-sixth of the concentration of the diamidine used by Lacey, while the growth of *H. pertussis* and *H. paraptussis* was practically unaffected. As a result of this finding we prepared a meat extract medium containing the diamidine and penicillin and tested its value for the primary isolation of *H. pertussis*.

EXPERIMENTAL

Bordet-Gengou medium

A mixture of 100 g. thinly sliced potato, 8 g. glycerol and 192 ml. distilled water was steamed for 2 hr. and filtered through lint. The extract was added to 8 g. agar (Davis Standard—from Thomson, Skinner & Hamilton Ltd., 12 Cadogan Street, Glasgow, C.2) dissolved in 600 ml. 0.6% NaCl and sterilized by autoclaving at 10 lb./sq.in. for 15 min. Finally 45 ml. of defibrinated rabbit blood and 0.9 ml. of a solution of penicillin G (50 units/ml.) were added to each 100 ml. of the basal medium.

Meat-extract medium

Lean horse meat (best steak; 500 g.) was minced and steamed for 2 hr. with 1 l. distilled water containing 6 g. NaCl. The extract was filtered hot through filter-paper (Whatman no. 1) and to the clear fat-free filtrate (1000-1050 ml.),

10 g. agar (Davis) was added. The pH was adjusted to 7.8 and the basal medium so obtained sterilized by autoclaving at 10 lb./sq.in. for 15 min. After cooling to 50°, 25 ml. oxalated horse blood (Burroughs Wellcome & Co.), 0.75 ml. of penicillin G. solution (50 units/ml.), and 0.3 ml. of a 0.1 % (w/v) solution of 4:4'-diamidino-diphenylamine-dihydrochloride (M. & B.938) were added to each 100 ml. of basal medium.

It is important in the preparation of this medium to ensure that it is free from fat which is inhibitory to *Haemophilus pertussis*. The hot filtration through filter-paper is usually adequate if all gross fat is removed from the meat before mincing. If, however, the filtrate does contain fat globules it should be allowed to stand overnight at about 4° and the solidified fat particles then removed by a further filtration.

RESULTS

Two hundred and sixty-four pernasal swabs from children suspected of whooping cough were cultured on Bordet-Gengou medium (BG) and on the selective meat-extract medium (ME) in parallel. At first each swab was inoculated on a 2½ in. diam. Petri dish of each medium, and these dishes were used throughout for BG medium. The greater selectivity of the ME medium, however, made it possible in the later stages of the work to use only one-half of a 2½ in. plate or one-quarter of a 3½ in. plate without any apparent loss of efficiency. After inoculation the plates were incubated for 4 days at 37°.

From 264 pernasal swabs examined in this way, *Haemophilus pertussis* was isolated on thirty-eight occasions on BG, and fifty-two occasions on ME. Growth of organisms other than *H. pertussis* was obtained on 49 % of the BG plates inoculated. With ME medium the growth other than *H. pertussis* was usually confined to a small number of colonies of diphtheroid or coliform organisms. That this greater selectivity was achieved without detriment to *H. pertussis* is suggested by the observation on twenty plates that the average colony diameter of the organism was 0.55 mm. on BG, and 0.60 mm. on ME medium. There was no evidence, however, that growth of *H. pertussis* was more rapid on ME than on BG.

Haemophilus paraptussis was shown to grow quite well on the ME medium, and although isolated only twice during the comparative tests has also been isolated on several occasions on ME when the BG medium was not regularly in use. As with *H. pertussis* growth was observed after 2 or 3 days.

CONCLUSIONS

The advantages of this selective medium containing horse-meat extract, horse blood, salt, penicillin and 4:4'-diamidino-diphenylamine are: (1) it is relatively easy to prepare; (2) only 20 % of sterile oxalated horse blood is required; (3) it is highly selective since significant growth of organisms other than *Haemophilus pertussis* and *H. paraptussis* seldom occurs, and it is not inhibitory to these latter organisms; (4) the percentage of positive isolations exceeds that obtained with the Bordet-Gengou medium; (5) the medium is a reliable one for the isolation of *H. paraptussis*.

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REFERENCES

- DAWSON, B., FARNWORTH, E. H., MCLEOD, J. W. & NICHOLSON, D. E. (1951). Observations on the value of the Bordet-Gengou medium for the cultivation of *Haemophilus pertussis*. *J. gen. Microbiol.* 5, 408.
- LACEY, B. W. (1951). Selective media for *Haemophilus pertussis* and *H. paraptussis*. *J. gen. Microbiol.* 5, vi.

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