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Selective Medium for *Corynebacterium equi* Isolation

JOHN B. WOOLCOCK,* ANNE-MARIE T. FARMER, AND MALCOLM D. MUTIMER

Department of Veterinary Pathology and Public Health, University of Queensland, St. Lucia, Queensland 4067, Australia

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The development of a selective medium for the isolation of *Corynebacterium equi* is described. The medium has been used to examine fecal samples from 127 horses of which 90 have been found to carry the organism.

Corynebacterium equi is an important pathogen of young horses, being associated with a purulent bronchopneumonia and, frequently, an enteritis. The same organism may also be recovered from porcine cervical lymph nodes and less commonly from bovine cervical nodes (R. A. McKenzie and B. A. Donald, J. Comp. Pathol., in press). Furthermore, there has been an increasing number of reports of human infection due to this organism (1, 5, 6). Precise knowledge of the epidemiology and epizootiology of *C. equi* is limited, and much of the available information is speculative. Although the infection in horses is sporadic, on some properties a number of animals can be affected. An adequate explanation for this behavior of the organism is dependent on a proper knowledge of its habitat. We considered the possibility that if *C. equi* was present in the soil (4), it might also be present in horse feces. The development of a selective medium for isolation of *C. equi* in mixed culture was therefore undertaken.

We began by evaluating and modifying a selective medium which has been used extensively for corynebacteria (FTO agar) and which contains a nitrofuran (Furoxone), Tween 80, and oil red O added to Trypticase-soy, yeast extract agar (7). The colonial morphology of *C. equi* on this medium was markedly different to that on sheep blood agar plates (blood agar base, Difco, supplemented with 7% sheep blood). Removal of the Tween 80 resulted in improved but still unsatisfactory growth. It was therefore decided to study the effect of varying the antibiotic and the dye component of the medium. Minimal inhibitory concentrations for 12 strains of *C. equi* were determined for the following antibiotics, using the replica plate procedure with concentrations ranging from 1 to 100 μg/ml: penicillin G (sodium), ampicillin, methicillin, neomycin, streptomycin, chloramphenicol, tetracycline, furazolidone (Furoxone), erythromycin, lincomycin, spiramycin, and spectinomycin. Results of the determinations indicated that furazolidone at 30 μg/ml would be satisfactory for incorporation in a selective medium. For all other antibiotics tested, minimal inhibitory concentrations were less than 10 μg/ml, and we considered this level too low to inhibit bacteria likely to be encountered in the feces. From the work of Hagedorn and Holt (3) who used dye-containing media for differentiation of a number of soil-borne organisms, a number of dyes were selected. Bromothymol blue (30 to 150 μg/ml), bromocresol purple (30 μg/ml), malachite green (10 μg/ml), nile blue (2.5 μg/ml), and eosin Y (50 μg/ml) were incorporated separately in the base medium (pH 7.2), consisting of 30 g of tryptone soya broth, (Oxoid Ltd.), 1 g of yeast extract powder (Oxoid), 15 g of agar (Difco), and 1,000 ml of distilled water, and tested with 12 strains of *C. equi*. Results indicated that bromothymol blue at 50 μg/ml would be satisfactory for isolation of the organism. When the combination of Furoxone and bromothymol blue was added to the base medium, all 12 strains tested grew, but the mucoid, luxuriant growth characteristic of this organism was not apparent. Removal of the furazolidone eliminated this problem, but the presence of bromothymol blue alone was insufficiently selective for *C. equi* in a mixed bacterial population. As FTO agar and its modifications were unsatisfactory for this *Corynebacterium*, alternative formulations were tried.

Two further antibiotics were tested for their minimal inhibitory concentrations against 12 strains of *C. equi*, namely, nalidixic acid (1 to 100 μg/ml) and novobiocin (0.75 to 25 μg/ml). The former was chosen because of its known effectiveness against gram-negative organisms and its utilization in a number of other selective media. Novobiocin was considered a possibility because it has a penicillin-like range of activity, but does not affect *C. equi*. Because of the possibility of fungal overgrowth from feces, cycloheximide was also incorporated. Tellurite has traditionally been added to blood agar for the differentiation of *C. diphtheriae* and was also

added to our base medium in the hope of achieving the same effect with *C. equi*. The final composition of the medium (hereinafter, NANAT) was as follows: base medium (as above) supplemented by nalidixic acid (20 μg/ml), novobiocin (25 μg/ml), cycloheximide (40 μg/ml), and potassium tellurite (0.005%).

NANAT medium was next tested with a range of gram-negative and gram-positive bacteria, including some known to be present in equine feces: *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, *S. muenchen*, *Proteus vulgaris*, *Serratia marcescens*, *Alcaligenes faecalis*, and *Pasteurella multocida*. Organisms were plated separately onto NANAT medium, using an inoculum of 1×10^5 to 5×10^5 colony-forming units per ml. All but one of these organisms did not grow on the medium; *P. aeruginosa* was distinguished by its pigment production and rough, flat, spreading colonies with thin, irregular margins. *C. equi* retains its characteristic growth on this medium; colonies are of variable size, moist, and glistening, tending to become confluent and appear in various shades of grey. NANAT medium partially inhibits the growth of *C. equi*, with a recovery rate of 77% relative to the base medium and 87% relative to sheep blood agar (Table 1).

This medium has now been used to examine fecal samples from 127 horses. A small portion of each fecal sample was removed with forceps and spread over one-half of the plate. The remainder of the plate was used for streaking. Plates were incubated in aerobic conditions for 48 h at 37°C. Mixed growth most frequently resulted, but separated colonies were always obtained. The distinctive colony of *C. equi* could thus be recognized (Fig. 1) among the other organisms, of which *Candida* sp., coryneforms, and occasionally pseudomonads predominated. We have been able to recover *C. equi* from the feces of horses when samples were taken within a few hours of collection and when the same feces were left for 4 days at room temperature. We have never been able to detect *C. equi* in the feces of normal horses by using nonselective media. Results show that 90 of the 127 horses examined carried *C. equi* in their feces. (Identification of the organism was confirmed by the criteria of Cummins [2]). In situations where *P. aeruginosa* proves a problem in the recovery of *C. equi*, the incorporation of polymyxin B (30 μg/ml) into NANAT medium is advantageous.

TABLE 1. *Recoverability of pure cultures of C. equi on nonselective and selective media*

Medium	Total colony count	% Recovery
Sheep blood agar	1.56×10^8	88
Trypticase soy, yeast extract agar (base medium)	1.76×10^8	100
NANAT medium	1.35×10^8	77

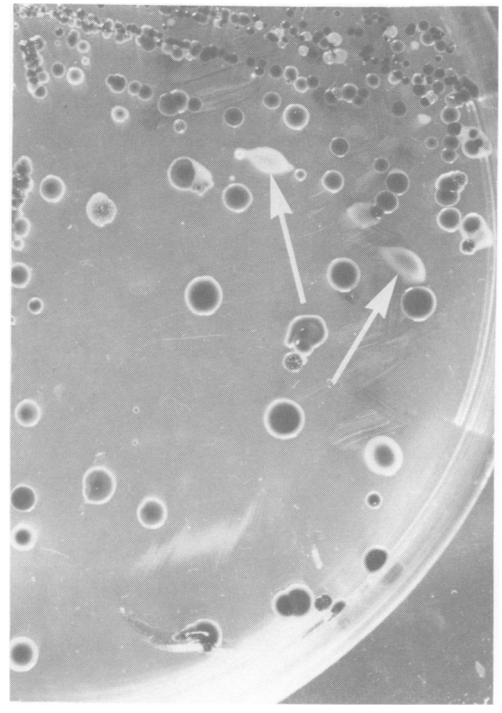

FIG. 1. *Growth, at 48 h, of C. equi (arrows) on NANAT medium inoculated with horse feces, showing the characteristic mucoid colonies.*

We have not evaluated NANAT medium for the examination of soil samples, but a selective medium for such a purpose is clearly warranted. However, the demonstration of *C. equi* in such a high proportion of horse feces suggests that some of the speculations relating to the epizootiology of the equine infection may need to be revised. Utilization of NANAT medium should help to clarify some of these issues. It may also be of value for investigation of human infections due to this organism.

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