Semiselective Medium for Isolation of \textit{Moraxella bovis} from Cattle with Infectious Keratoconjunctivitis

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The incorporation of 2.5 μg/ml of cloxacillin into 5% bovine blood agar provided an inexpensive, easily prepared culture medium for the primary isolation of \textit{Moraxella bovis} from bovine lacrimal and nasal secretions. With this medium, the time required to identify and isolate \textit{M. bovis} from large numbers of field specimens was substantially reduced, whereas the sensitivity of isolation was increased by 60%.

\textit{Moraxella bovis} is the infectious agent most frequently isolated from cases of infectious bovine keratoconjunctivitis (1-5). However, a large number of potentially pathogenic and commensal bacteria constitute the normal flora of both healthy and diseased eyes (6). These can rapidly overgrow the slower growing \textit{M. bovis}, with the result that the sensitivity of isolation procedures is diminished and considerable time is spent in subculturing from primary isolation plates to obtain pure cultures of \textit{M. bovis}.

The purpose of this study was to develop and evaluate a semiselective medium for the primary isolation of \textit{M. bovis}, containing cloxacillin, to which all 66 hemolytic and 18 nonhemolytic isolates of \textit{M. bovis} were found to be uniformly resistant (7).

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Brain heart infusion agar (GIBCO Diagnostics, Madison, Wis.), supplemented with 5% defibrinated bovine blood (BA), was prepared by a standard technique (4). Cloxacillin blood agar (CBA) was prepared by adding sodium cloxacillin (Sigma Chemical Co., St. Louis, Mo.) to sterile BA that had cooled to 42°C for a final concentration of 2.5 μg of cloxacillin per ml of BA. The CBA was then dispensed into sterile disposable polystyrene petri dishes (15 by 100 mm) and stored in sealed containers at 4°C.

 Conjunctival swab specimens were secured from a herd of cattle undergoing an epizootic of infectious bovine keratoconjunctivitis. Samples were taken from the ventral conjunctival sac of healthy and diseased eyes. A total of 35 swabs were obtained and transferred immediately to plates. One side of the swab was used to streak one-third of the surface of a BA plate; the swab was then rotated 180 degrees and the opposite side used to streak one-third of the surface of a CBA plate. Plates were stored in an ice chest for transport back to the laboratory, where they were each further streaked with a sterile platinum loop to obtain isolated colonies. After incubation at 35°C for 24 h, the growth on the BA and CBA plates was evaluated and compared.

The number of different bacterial organisms, based on colony morphology differences after 24 h of incubation, was counted and recorded for BA and CBA plates. Isolation of \textit{M. bovis} was confirmed by examination of smears by fluorescence microscopy (3; J. J. W., Ph.D. dissertation).

\textit{Moraxella bovis} was isolated from 16 (45.7%) of the CBA plates and from 10 (28.6%) of the BA plates. In no instance was \textit{M. bovis} isolated from a BA plate and not a CBA plate. In addition, significantly \((P < 0.01)\) fewer colony types were isolated on CBA plates (mean, 1.4) than on BA plates (mean, 4.1) (2; Fig. 1).

The incorporation of cloxacillin (2.5 μg/ml) into standard 5% bovine blood agar increased the sensitivity of our culture and isolation of \textit{M. bovis} from clinical specimens by 60%. When large numbers of field specimens are being processed, not only is there a considerable amount of time spent in identification of the correct colony types, but also in subculturing in order to obtain pure cultures of \textit{M. bovis}. The almost pure cultures of \textit{M. bovis} obtained represent a significant saving in time and material costs.

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FIG. 1. Demonstration of CBA for the isolation of *M. bovis*. Plate a is 5% bovine blood agar (brain heart infusion base) streaked with a swab from an eye with acute infectious bovine keratoconjunctivitis; discrete colonies of *M. bovis* are not readily discernible. Plate b is 5% bovine blood agar containing 2.5 µg of cloxacinil per ml and was streaked with the same swab as plate a. *M. bovis* is present in almost pure culture.

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