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Isolation of *Mycoplasma dispar* from Cattle in the United States

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Mycoplasma dispar was first isolated from pneumonic calf lungs and described by Gourlay and Leach (1970). This mycoplasma has certain characteristics, such as colony formation and fastidious medium requirements, similar to those of *Mycoplasma hyopneumoniae*. Inoculation of *M dispar* by the intratracheal route has been shown to result in a proliferative interstitial pneumonia in caesarean-derived or colostrum-free calves (St George and Horsfall 1973). Cultures of lung, tracheal and bronchial tissue from nonpneumonic (macroscopic), three-month-old cattle yielded a greater than 50% recovery of *M dispar* (Thomas and Smith 1972).

This report concerns the isolation of *Mycoplasma dispar* from calves in the United States. The first isolate, referred to as 1606, has been made from grossly pneumonic lung tissue from a 90 kg crossbred (Charolais, Angus, Brahma) calf shipped to Greenfield, Indiana, from Florida in July of 1974. Serial tenfold dilutions of triturated pneumonic tissue were made in a liquid medium suitable for growth of *M hyopneumoniae* and in the broth medium for *M dispar* as described by Gourlay and Leach. Ampicillin instead of benzylpenicillin was added to the liquid media at a concentration of 500 mcg per ml. These liquid media were incubated at 39°C in tightly stopped glass tubes. In addition, solid medium prepared by the addition of Epiagar* to the corresponding broth media was inoculated and incubated at the same temperature in a moist chamber with 5% carbon dioxide. Centreless mycoplasma colonies with a slightly granular or lacy appearance when viewed with transmitted light were observed following three to four days' incubation. Attempts to subculture single colonies from solid to liquid phase media were unsuccessful. However, subculturing from liquid to liquid medium was possible and colonies were observed on agar media following each transfer. These colonies were considerably smaller, even upon dilution, than those observed on primary isolation. Growth of the mycoplasma in liquid media was accompanied by the production of acid. On primary isolation, the pH change required several days; as the isolate became adapted to synthetic media, the change was usually detectable within 24 to 48 hours. In later work, foetal calf serum was added in place of swine serum in the *M hyopneumoniae* medium. No growth was detected with this isolate in several other media designed for propagation of more conventional mycoplasma.

This mycoplasma isolate has been serologically identified as *M dispar* by Dr. C. J. Howard at the Institute for Research on Animal Diseases, Compton, Newbury, Berkshire, England. Antiserum against *M dispar*-type culture 462/2 had a titre of greater than 1280 in a metabolic inhibition test against isolate 1606. Titres with *M bovirhinis* PG43, *A laidlawii* 1305 antisera and normal rabbit serum were all less than 20 against isolate 1606.

Mycoplasma with characteristics similar to *M dispar* isolate 1606 have been isolated from the lungs of two of ten, 55-kg Holstein calves located on a dairy farm in the south central area of California. The only gross lesions present in these calf lungs were small dark areas of atelectasis confined to the right apical lobe in one calf and the intermediate lobe of the other. The identity of these California isolates will be confirmed serologically. Two additional calves from the same herd yielded colonies similar to *M dispar* but growth could not be initiated in liquid medium.

Studies are under way in our laboratory involving the culturing of pneumonic calf lungs for *M dispar* from several additional geographical areas in the United States. The probability is high that this agent is as widely disseminated in cattle in this country as has been reported in England and in Australia.

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