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Pasteurella multocida

A Study on the Isolation, Identification and Characterization of New Zealand Strains.

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ABSTRACT

Pasteurella multocida is a small Gram negative bacillus which causes disease in many animal species. Its pathogenicity is attributed to two mechanisms: invasiveness and toxin production. The proportion of strains which produce toxin is low but such strains cause severe atrophic rhinitis in swine. Invasiveness is the predominant mechanism of pathogenicity and is largely dependant on the possession of a capsule.

P. multocida is divided into five serotypes (A, B, D, E and F) based on the antigenic structure of the capsule and a correlation exists between serotype, disease and the animal species affected. However, many isolates are non-typable due to the possession of a non-antigenic (hyaluronic acid) capsule. The presence of an antigenic capsule and a hyaluronic acid capsule are independant. *ie* one, both or neither may be present.

To facilitate the isolation of *P. multocida* we assessed the selective medium of Smith and Baskerville(1983) and found that many New Zealand isolates of *P. multocida* grew poorly in the absence of blood (not present in Smith and Baskerville's medium). Furthermore, many isolates were inhibited by Polymixin and the high alkalinity (pH 8.6) of the medium.

On the basis of these observations we formulated a modified selective medium which omitted Polymixin and was composed of a blood agar base at neutral pH. However, it contained three antibiotics (Gentamycin, Bacitracin and Mycostatin) present in the original medium of Smith and Baskerville. This modified medium propagated all our test isolates of *P.multocida* which were derived from seven

animal species (fowl, sheep, goat, cattle, rabbit, dog, cat). It also suppressed the background flora present in swabs taken from the nasal cavity of rabbits.

Both the traditional isolation technique of plating specimens on blood agar and the modified selective medium were used to obtain isolates of *P. multocida* from several species of domestic animal. These were: pigs (15 isolates), rabbits (25), cats (10), dogs (21) and deer (1). Isolates were examined in some detail.

Isolates were serotyped using the indirect haemagglutination assay (IHA). They were found to be Type A (7%), B (1%), D (9%) or untypable (83%). This is similar to overseas findings.

The IHA assay is a laborious technique so two possible alternatives were examined. *viz* sodium dodecyl sulphate polyacrylamide-gel analysis (SDS-PAGE) of proteins and restriction endonuclease analysis (REA) of DNA. Both techniques failed to distinguish between *P. multocida* serotypes in the sense that isolates were extremely heterogeneous and most gave a unique pattern both with SDS-PAGE and REA.

This heterogeneity allowed the identification of individual strains of *P. multocida* and consequently SDS-PAGE was used in an epidemiological investigation which traced the origin of an outbreak of respiratory disease in rabbits and provided evidence that the outbreak was not (as was earlier believed) due to the introduction of rabbits from overseas into a New Zealand colony. Furthermore, using this approach we were able to show that strains which cause severe pneumonia were not confined to the lower respiratory tract but could also be carried in the nasal cavity. This is consistent with the accepted hypothesis that *P. multocida* is an opportunistic pathogen.

Severe atrophic rhinitis is a disease of pigs which is caused by toxin-producing strains of *P. multocida*. The disease is responsible for much economic loss overseas but has not been reported in New Zealand. We obtained a toxigenic strain of *P. multocida* and used it to validate an *in vitro* (mammalian cell culture) method for detecting toxin-producing strains. This method was then used to examine New Zealand isolates of *P. multocida*. None of these were shown to be toxigenic. This suggests that toxigenic strains, if present in New Zealand, are not common.

Diseases due to *P. multocida* are commonly treated with antimicrobial agents such as the Penicillins, Aminoglycosides and Sulphonamides. Strains which are resistant to these agents are present in overseas countries and resistance is associated with the presence of plasmids.

We determined the minimum inhibitory concentrations (MIC), of four antimicrobials (Penicillin G, Streptomycin, Tetracycline and Sulphadiazine) for New Zealand isolates. Considerable variation was found between isolates but no isolate was of sufficiently high resistance to be considered "resistant". This was despite the fact that 12 of 73 (16%) of the field isolates possessed plasmids of a similar size (1Mdal to 5Mdal) to plasmids known to carry antibiotic resistance markers.

Despite its importance as a pathogen of swine, cattle, sheep, fowl and rabbits, *P. multocida* has been little studied in New Zealand. This thesis represents a preliminary stage to a fuller understanding of the importance of *P. multocida* in this country and the best means of control. The possibility of outbreaks of disease due to *P. multocida*, *eg* atrophic rhinitis, may well stimulate further work on this organism.

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