THE CHARACTERISATION OF STRAINS OF *MYCOPLASMA OVIPNEUMONIAE* BY RESTRICTION ENDONUCLEASE ANALYSIS

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Alison Jane Mew

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ABSTRACT

Studies of the pathogenicity of *Mycoplasma ovipneumoniae* would be facilitated by an *in vitro* method of identifying and classifying isolates of this organism, and it is with the development of such a method, and its possible applications, that this thesis is mainly concerned.

The method chosen was Restriction Endonuclease Analysis (REA) which has already been successfully applied to the identification of some viruses and bacteria.

Initially the REA patterns of 2 strains of *M. ovipneumoniae* were observed. They differed markedly in restriction pattern, but the pattern exhibited by any one of them was recognisable and reproducible. Furthermore, patterns did not change significantly after limited passage *in vitro*. An extension of this study to 8 isolates from widely differing sources, showed that all gave markedly different patterns. It was concluded that, unlike the findings for *Leptospira* and *Rhizobium*, REA could not be used for the identification of species, but is an extremely powerful method for identifying strains of *M. ovipneumoniae*.

Despite the marked heterogeneity of isolates from different sources, the relative stability of pattern of an individual isolate, suggested that REA could be used to examine the epidemiology of individual strains of *M. ovipneumoniae* within a flock of sheep. Hence, we undertook a study of *M. ovipneumoniae* isolates obtained by serial swabbing of the nasal cavities of a flock of lambs over a 6-month period, and from the lungs of the same lambs at slaughter. It was shown that 54 isolates from the nasal cavities fill into 7 major groups with respect to restriction pattern (although minor differences were detected within a group). There was a tendency for these groups to occur sequentially. None of the isolates were shown to persist for long periods, but a later strain could replace an earlier one. The isolates from the lungs were more homogeneous and the predominant strain fell within one of the 7 "nasal" groups. This suggests that nasal isolates

may vary in their pathogenicity for the lungs, although other explanations are possible (see General Discussion).

Notwithstanding the apparent stability of mass cultures of M. ovipneumoniae following limited passage in vitro, the unexpectedly large number of restriction patterns found with field strains, led us to re-examine, in more detail, the stability of cloned isolates. A multiply-cloned isolate was propagated in vitro and 8 sub-clones selected before and, a further 8 sub-clones after 20 passages. Some limited heterogeneity was detected among the 8 sub-clones selected before passage, and a somewhat greater degree of heterogeneity was detected among sub-clones selected after passage. It should, however, be emphasised that these differences were small compared to the total lack of similarities seen when isolates from different sources were examined. Limited passage in the presence of sub-lethal concentrations of antibody did not increase the heterogeneity of patterns - if anything, the reverse is true.

Explanations for these findings and future experiments to confirm or deny these possibilities are discussed.

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