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STUDIES OF *Pasteurella haemolytica*:

(i) COMPARISON OF SEROTYPING TECHNIQUES

(ii) PREVALENCE OF SEROTYPES IN NEW ZEALAND SHEEP

A THESIS PRESENTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE IN MICROBIOLOGY AT
MASSEY UNIVERSITY, NEW ZEALAND

DIANE VALERIE PRINCE

1985

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ABSTRACT

P.haemolytica, which is the aetiological agent of pasteurellosis, is the major cause of mortalities in sheep in Great Britain and is a problem in several other countries including New Zealand. Furthermore, P.haemolytica, acting as a secondary invader, exacerbates lesions of chronic non-progressive pneumonia (CNP) initiated by Mycoplasma ovipneumoniae and this disease causes considerable economic loss to the New Zealand sheep farming industry.

P.haemolytica exists as 15 serotypes and immunity is serotype specific. P.haemolytica vaccines are marketed overseas and their use in New Zealand is under active consideration. It is logical however, to establish which serotypes are present in New Zealand before a vaccine is produced, but there is at present, no information on this point. This is largely because of technical difficulties in typing isolates. This situation stimulated the present investigation which has 2 major aims: To develop an improved method of typing P.haemolytica and to gather some data on the prevalence of the various serotypes in New Zealand.

With respect to the first aim, several approaches were taken to the problem of differentiating between serotypes.

(1) Indirect heamagglutination (IHA), the standard method by which P.haemolytica is serotyped, was found to be laborious and gave many cross-reactions.

(2) SDS-PAGE of total protein showed similar patterns for all

serotypes within a biotype, whereas the 2 biotypes had different patterns. Thus, SDS-PAGE cannot be used to identify the serotype, but could be useful for identifying the species and the biotype.

(3) Latex beads, coated with antibody prepared against whole cells, agglutinated homologous cells, but also gave many cross-reactions. This test should, in principle, become type-specific if purified capsule were used as the immunising antigen, but further work is required to prepare capsular polysaccharide free from endotoxin which stimulates the production of cross-reacting antibody.

(4) Gel precipitation was simple to perform and was serotype specific. However, serotype A2 required a concentrated antigen preparation for the detection of a precipitation line. The concentrated antigen could not be routinely used with the other types because of cross-reactions between antigens.

(5) Counter immunoelectrophoresis (CIE) was not serotype specific due to endotoxin causing cross-reactions.

(6) Bacterial agglutination was a rapid and simple test to perform and showed some limited cross-reactions.

Since IHA, gel precipitation and agglutination showed some potential, they were then compared using 40 isolates from the lungs of 60 sheep with CNP These findings reinforced the conclusions drawn when prototype strains were used.

We initially proposed that the serotype of isolates should

be primarily determined by agglutination tests, but since this is not 100% specific, the results must be confirmed by gel precipitation. This approach was investigated using 110 isolates from the nasopharynx of sheep and it was satisfactory for all serotypes except A2. Strains within the A2 serotype showed some heterogeneity at least when examined by gel precipitation and a hypothesis is presented to explain this.

We conclude that at present, the best method of serotyping isolates is by gel precipitation, but that all isolates which are not positively serotyped by this approach should be retested by IHA.

Information concerning the prevalence of serotypes was obtained during the above studies. 110 isolates were obtained from the nasopharynx of 50 sheep from each of 4 farms. All "A" serotypes, except A12 and A14, were isolated. Serotype A2 made up 35% of these isolates. 60 lungs with CNP lesions were obtained from 20 farms in the Manawatu region and 40 isolates of P.haemolytica were obtained. All were serotyped and they were: A1 (25%), A2 (55%), A6 (7.5%), A7 (7.5%), A8 (2.5%), and A9 (2.5%). No "T" types were found in either the nasopharynx or the lungs. The implications of these findings for vaccine production are discussed.

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