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STUDIES ON *PASTEURELLA HAEMOLYTICA*: COMPARISON OF SEROTYPING TECHNIQUES AND SURVEYS OF THE PREVALENCE OF SEROTYPES IN SHEEP AND GOATS IN NEW ZEALAND

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

ANNE CAMILLA MIDWINTER

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ABSTRACT

P.haemolytica is the aetiological agent of pneumonic pasteurellosis in sheep and goats, and, as a secondry invader it also exacerbates lesions of chronic non-progressive pneumonia (CNP). These diseases cause considerable economic loss to the New Zealand farming industry.

P.haemolytica exists as 15 serotypes and immunity is serotype specific. Vaccines against *P.haemolytica* are produced, but it is not known if the serotypes contained in the vaccine are the same as those causing disease in New Zealand as there is a lack of information on the prevalence and distribution of the serotypes of *P.haemolytica* in this country. This is largely due to the technical difficulties involved in typing isolates because the standard method, the indirect haemagglutination assay, (IHA), is laborious and may give anomalous results due to cross-reactions.

The present investigation was undertaken with two major aims: to replace IHA with a more convenient typing system, *viz.* agar gel immunodiffusion, (AGID), and to use AGID to survey the serotypes of *P.haemolytica* present in CNP lesions of sheep, pneumonic pasteurellosis of sheep, pneumonic pasteurellosis of goats and the nasal cavities of goats.

Difficulties were encountered in the preparation of rabbit antisera to some of the 15 prototype strains. These difficulties were overcome by using domestic hens when necessary. Using these sera it was possible to distinguish the 15 prototype strains by IHA, and following absorption of sera, by AGID. The results obtained by IHA and AGID were in agreement, at least when prototype strains were examined. It was necasary to show that AGID is able to correctly establish the serotype of field isolates of

P.haemolytica. Hence 25 caprine isolates of *P.haemolytica* from field cases of pneumonic pasteurellosis were serotyped by both IHA and AGID. In 24 cases the results from the two tests agreed. In the remaining case IHA indicated that the isolate was serotype A2 or A11. We were able to show that this isolate gave a line of identity with antigen prepared from the prototype strain of A11, but showed no line of identity in the AGID with any other antigen preparation. Taking this as the critical criterion we concluded that this isolate was serotype A11, although IHA showed a 2-fold preference for A2 over A11. Since AGID was shown to be a reliable test we used it alone for future serotyping, for two reasons: it is more convenient, and any cross-reactions that do occur may be resolved by looking for a line of identity between antigens of the isolate and a prototype antigen. In the case of two serotypes involved in many cross-reactions, namely A1 and A7, the capsular polysaccharide was purified by organic solvent precipitation. This purified polysaccharide was used to test for a line of identity with reacting isolates. This eliminates the possibility that the line of identity seen was due to a non-serotype-specific antigen.

Four surveys (two in sheep, two in goats) of the serotypes of *P.haemolytica* present in New Zealand were undertaken. The first involved 139 isolates derived from ovine lesions of CNP collected from 4 areas of New Zealand. A total of 9 serotypes were found. Serotypes A1 (31.7%), A2 (47.8%) and A7 (10%) made up 89.5% of the total.

A smaller survey of 18 isolates from pneumonic pasteurellosis of sheep revealed 6 serotypes, including 1 isolate of T10, a serotype and biotype not previously found in New Zealand. A1 (11.1%) and A2 (61.1%)

were the predominant serotypes present and represented 72.2% of the total.

The 25 isolates of *P.haemolytica* from caprine pneumonic pasteurellosis contained only 4 serotypes. A2 represented 80% of the total.

14 isolates of *P.haemolytica* were obtained from the nasal cavities of 109 goats. Only 2 serotypes were isolated. 13 isolates were A2 and the remaining isolate was A11.

The implications of these results for vaccine manafucture were discussed and it was suggested that a vaccine containing A2, A1 and A7 (in order of importance) should control CNP in sheep and pneumonic pasteurellosis in both sheep and goats.

Field isolates of *P.haemolytica* were compared with prototype strains for capsule production (using Laurell Rocket test), and antibiotic sensitivities. The total proteins of caprine and ovine strains were also compared, using SDS-PAGE. Laurell Rocket tests showed that the prototype strains produced more capsular polysaccharide than did any of our field isolates. All isolates of *P.haemolytica* showed some resistance to streptomycin while none were resistant to more than 4µg/ml chloramphenicol or penicillin so these are the drugs of choice. No difference was found within a serotype between the total proteins of caprine and ovine isolates by SDS-PAGE.

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