Bacteriological Studies of *Dichelobacter Nodosus* Isolated from Footrot Infected Sheep*

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Introduction

Footrot is a contagious disease of ruminants particularly sheep and goats although cattle and deer may also be affected (Beveridge et al., 1941). The disease has been known for the last two centuries in many parts of the world, however, it has only been detected in Malaysia in the last five years. The first case of footrot occurred in Institut Haiwan Kluang (IHK) in 1994 and becoming an increasingly important disease in Malaysia. Vaccination with commercial vaccine did reduce the prevalence but the strength and duration of the immunity achieved is limited (Egerton et al. and Meritt, 1973). Extensive studies are being carried out on footrot disease to obtain a comprehensive knowledge about the etiological agent and to understand the situation in the country.

Main objectives of the project were to characterise *Dichelobacter nodosus* found in Malaysia and to explore the possibility of developing a serogroup specific vaccine of high potency. This involves the production of fimbriae by recombinant DNA in a host that is less fastidious growth requirement and is able to grow to a much higher density than *Dichelobacter nodosus*. Apart from that, to study epidemiology of the disease in Malaysia as well, the pathogenesis of the bacteria in causing the disease.

Materials and Methods

Isolations of *Dichelobacter nodosus* were made from several sheep farms in Malaysia. The isolates obtained were identified and confirmed as *D. nodosus* by polymerase chain reaction using spesies specific primers. These isolates were subjected to laboratory tests in order to characterise and differentiate them. Two conventional tests i.e. elastase and gelatin gel tests (Link and Morris, 1996) were used to assess the virulence of the isolates. Molecular techniques, which include pulsed field gel electrophoresis analysis, were also done to further differentiate the isolates. The isolates were also tested on their susceptibility to different antimicrobial agents. In developing specific recombinant vaccines, detail studies were done on the fimbrial gene of the organism. The fimbrial gene of the isolates was detected by PCR technique. The *D. nodosus* fimbrial gene were then cloned and expressed in another host i.e. *Pseudomonas aeruginosa*. Antigenicity of the recombinant vaccine was determined by Western Blot.

Epidemiology studies were carried out at three farms for one year (twelve visits each farm). Footrot lesions were scored and sampled for *D. nodosus* isolations and characterisation. Pathogenicity studies were done according to gross lesion scores. Skin biopsies were taken for pathological studies by light and electron microscope.

Results and Discussion

Twelve *Dichelobacter nodosus* were isolated from 30 sheep with footrot lesions. Diagnosis was done successfully by Gram-stain method while polymerase chain reaction (PCR) method confirmed the isolates as *Dichelobacter nodosus* by producing a single product of approximately 780 basepairs. All isolates, although obtained from distant locations, were of serogroup B. Among the antibiotics tested, penicillin G proved to be the most effective antibiotic with $MIC_{90\%}$ of 0.023 µg/ml. Generally, the isolates exhibited variation in the laboratory characteristics although they had been isolated from the similar lesion score. Some of the isolates, which appeared to have the capability of causing virulent footrot *in-vitro*, failed to show clinical signs of virulent form of footrot . This was probably due to the frequent topical regimen adhered to and results of the vaccination programme by the farm management. All isolates were found not to contain plasmid by standard plasmid extracting method. This indicates that the genes coding for virulence of the isolates were not palsmid-mediated. Molecular typing of the isolates was successfully carried out by pulsed field gel electrophoresis analysis. Significant patterns were generated by three GC-rich enzymes discriminating the isolating into eight genome types. Isolates from the same flock were also shown to possess variation in their PFGE profiles.

The gene coding for fimbriae was successfully amplified and cloned in a T-vector. An expression vector was chosen based on the fimbrial gene orientation and appropriate restriction enzymes were selected in order to design a recombinant plasmid. The fimbrial gene was recloned into the expression vector downstream the lac promoter. The plasmid containing the *D. nodosus* fimbrial gene was then successfully transformed in the surrogate host, *Pseudomonas aeruginosa*. Electrophoretic and western blot analysis showed that *Pseudomonas aeruginosa* containing recombinant plasmid (with *D. nodosus* fimbrial gene) expresses *D. nodosus* fimbriae instead of its own fimbriae. Preliminary tests were done by injecting these purified recombinant fimbriae in sheep. Results showed that there were high levels of antibody production against *D. nodosus*.

Conclusions

Dichelobacter nodosus isolates in Malaysia that belong to a single serogroup (serogroup B), are variable in their capability of causing different degrees of footrot. The isolates also demonstrate multiple genotypes even though they were obtained from the same flock. The development of specific footrot vaccine looks very

promising as we have already succeeded in expressing *D. nodosus* fimbriae in a surrogate host, *Pseudomonas aeruginosa* that is less fastidious and fast growing. This specific vaccine, which has commercial value worldwide, will be able to control and prevent the disease.

Benefits from the study

Results from this project will have significant impacts for the animal industry in particularly sheep industry in Malaysia, and especially the production of serogroup specific vaccine. The specific vaccine will be able to elicit high level of antibody production in sheep thus protecting sheep from footrot disease. One master student had completed her study under this project. At present, there are two Ph.D. candidates pursuing their degrees in this field of study.

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