Bacteriological, Pathogenicity, Epidemiological and Control Studies of Ovine Footrot

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Introduction

Footrot is a contagious disease of ruminants particularly sheep and goats although cattle and deer may also be affected. The disease has been known for the last two centuries in many parts of the world, however, 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. 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 are 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 *Dichelobacter 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 were used to assess the virulence of the isolates In developing specific recombinant vaccines, detail studies were done on the fimbrial gene of the organism. The fimbrial gene of the isolates were 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

Fifteen *D. nodosus* isolates were recovered from 38 sheep showing clinical signs of footrot in 2 government sheep farms located approximately 200 km apart. The isolates were studied and results analysed. Preliminary identification of the organism was carried out by the Gram-stain method while the polymerase chain reaction (PCR) method using species-specific primers, A and Ac, was employed for species confirmation. All 15 isolates produced a single product of approximately 780 basepairs. Although obtained from two different locations, all isolates were found to be of serogroup B. Two conventional methods, namely the elastase and gelatin-gel tests, were used to assess the virulence of the isolates. Generally, the isolates exhibited variations in the laboratory characteristics. Based on the virulence assessment, some of the isolates appeared to have the capability for causing virulent footrot but were isolated from sheep that did not show clinical signs of the virulent form of footrot. This was probably due to the constant topical treatment regime and the vaccination programme practised by the farm management which may have caused the bacteria to not fully express its virulence characteristics.

Analysis of the fimbrial subunit gene sequence revealed the local strains had sequences that are distinct from the prototype strains. There were 94 to 97 percent amino acid similarities (identities and conserved changes) between the local isolates and the prototype strains. The expression of *D. nodosus* fimbriae serotype B2 in an easily grown aerobe, *Pseudomonas aeruginosa*, were carried out successfully. *Dichelobacter nodosus* fimbrial subunit gene was cloned in an expression vector, pUCpKS downstream the *lac* promoter to construct the recombinant plasmid pMAL99. Recombinant *P. aeruginosa* cells containing this construct were able to produce a high yield of fimbriae. The fimbriae were physically, structurally and antigenically indistinguishable from those produced by the *D. nodosus* isolates from which the fimbrial subunit gene was originally derived. This was shown and confirmed by Western blot analysis. When the fimbriae produced by the *P. aeruginosa* harbouring pMAL99 were extracted, purified and used as vaccines in sheep, the results conclusively showed that these vaccines were equally effective as either the native whole cells or isolated fimbriae from *D. nodosus* in eliciting the antibody response. The vaccinated sheep were found protected against homologous serogroup challenge. The recombinant fimbriae also produced cross-protective antibodies to heterologous serotypes B3 and B4 infections. Therefore, the

monovalent serogroup specific recombinant vaccine has a good potential for use in farms in this country to protect sheep against footrot.

In the epidemiological study, four out of eight sheep farms investigated were found to be infected with *D. nodosus*. Five serogroups were identified comprising of B, A, C, F and I. Serogroup B was the predominant serogroup isolated (78.2%). Histopathological changes in virulent footrot were observed in the interdigital skin layers and hoof matrix ranging form acute dermatitis to hyperkeratosis, perakeratosis and acanthosis of the epidermis. In the SEM, a severe zone of lysis appearing as a surface depression around bacteria in the horny layer of the interdidgital skin of the hoof was detected in the virulent footrot, while this lesion was less severe in the benign form. TEM revealed degeneration in the epidermis and dermis. Immunohistochemistry observations validated the relationship between the lesions seen in footrot and virulence *D. nodosus*. Immunostaining reaction of benign footrot lesions was less intense than that of virulent footrot in the interdigital skin layers.

Conclusions

Dichelobacter nodosus isolates in Malaysia are variable in their capability of causing different degrees of footrot. The histopathology, immunohistochemistry and ultrastructural studies provided evidence that the virulence of *D. nodosus* caused interdigital dermatitis. The epidemiological studies have shown that environmental factors could encourage the spread of footrot disease. The development of specific footrot vaccine against the serogroup B (predominant serogroup in Malaysia) was successful as we have already succeeded in expressing *Dichelobacter nodosus* fimbriae in a surrogate host, *Pseudomonas aeruginosa* which is less fastidious and fast growing. This monovalent serogroup specific recombinant vaccine has a good potential for use in farms in this country to protect sheep against footrot since it was shown to have cross-protective antibodies to heterologous serotypes for serogroup B infections. This specific vaccine will be able to control and prevent the disease and has commercial value worldwide.

Benefits from the study

Results from this project will have significant impact for the animal industry in particularly sheep industry in Malaysia, 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. Two PhD student had completed their study under this project.

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable:

Nil

Project Publications in Refereed Journals

1. Zunita Z, Sheikh Omar AR, Mutalib AR, Son Radu, and Azmi ML. 2002. Analysis of fimbrial subunit gene of *Dichelobacter nodosus* isolated from footrot infected sheep in Malaysia *Asia Pacific J. Biotechnology and Molecular Biology. Vol 9(1):33-40.*

2. Zunita Z, Son Radu, Sheikh-Omar AR, Mutalib AR, Joseph PG, and Rusul G. 1998. Molecular analysis of *Dichelobacter nodosus* isolated from footrot in sheep in Malaysia. *Veterinary Microbiology. Jul: 62(3):243-250.*

3. Zunita Z, Sheikh-Omar AR, Mutalib AR, Joseph PG, and Son Radu. 1998. Virulent characteristics of *Dichelobacter nodosus* isolated from ovine footrot in Malaysia. *Malaysian Veterinary Journal 10(1), 5 - 9*

Project Publications in Conference Proceedings

1. Al-Jashamy K, Jasni S, Mutalib AR, Mohd Azmi ML, Zunita Z, Ramanoon SZ, and Sheikh Omar AR. 2001. Serology of *Dichelobacter nodosus* isolates from ovine footrot cases in Malaysia. InProceedings of The 24th Malaysian Microbiology Society Symposium, Kuantan, 9-12 September, 2001; p236-239.

2. Al-Jashamy K, Jasni S, Mutalib AR, Mohd Azmi ML, Zunita Z, Ramanoon SZ, and Sheikh Omar AR. 2001. Pathology of ovine footrot caused by virulent and benign strains of *Dichelobacter nodosus*. InProceedings of the 2nd International Congress/13th VAM Congress and CVA-Australasia/Oceania, Kuala Lumpur, 27-30 August, 2001; p132-135.

3. Al-Jashamy K, Jasni S, Sheikh Omar AR, Mutalib AR, and Ramanoon SZ. 2000. Ultrastructural studies of *Dichelobacter nodosus* morphology. In: Proceedings of the 9th scientific conference Electron Microscopy society of Malaysia,12-14 November 2000; p134-136.

4. Al- Jashamy K, Jasni S, Sheikh Omar AR, Mutalib AR, Mohd Azmi ML, and Ramanoon SZ. 2001. Epidemiological study of ovine footrot in Malaysia. 2001. In: Proceedings 2nd International Congress/13th VAM Congress and CVA-Australasia/Ocenia Regional Symposium; p129-131.

5. Al- Jashamy K, Jasni S, Mutalib AR, Zunita Z, Mohd Azmi ML, Ramanoon SZ, and Sheikh Omar AR. 2001. Serology of *D. nodosus* isolates from ovine footrot cases in Malaysia. 2001. Al- Jashamy K, Jasni S, Mutalib AR, Zunita Z, Mohd Azmi ML, Ramanoon SZ, and Sheikh Omar A.R. In: Proceedings of the 24th Malaysian Microbiology Society Symposium, Kuantan. 9-12 September, 2001; p73-76.

6. Al- Jashamy K, Jasni S, Mutalib AR, Mohd Azmi ML, Zunita Z, Ramanoon SZ, and Sheikh Omar AR. 2001. Pathological study of ovine footrot. In: Proceedings 2nd International Congress/13th VAM Congress and CVA-Australasia/Ocenia Regional Symposium; p132-135.

7. Al-Jashamy K, Jasni S, Sheikh Omar AR, Mutalib AR, and Ramanoon SZ. 2001. Immunogold labeling of *Dichelobacter nodosus* fimbriae. In: Proceedings of the 10th Scientific Conference, Electron Microscopy Society of Malaysia, 8-10 November, 2001; p242-245.

8. Zunita Z. 2001. Development of a recombinant vaccine against footrot disease in Malaysia. 2001. In: Recent Advances in Animal Health and Production no 24. 26 February, 2001; p16-17.

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12. Zunita Z, Mutalib AR, Sheikh-Omar AR. 1999. In-vitro antimicrobial susceptibility of *Dichelobacter nodosus* isolated from footrot in sheep in Malaysia. In: 3rd UNESCO National Workshop on Promotion of Microbiology in Malaysia, Mei 1999.

Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded *(e.g. M.SC/Ph.D.)*	Graduation Year *(or expected)*
Zunita Zakaria	**Molecular Analysis of *Dichelobacter nodosus* Isolated from Footrot Infected Sheep in Malaysia**	**Molecular Biology**	**Ph. D.**	**2002**
Karim Al-Jashamy	**Pathological, Bacteriological and Prevalance Studies of Ovine Footrot**	**Pathology**	**Ph. D**	**2003**

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