ISOLATION AND CHARACTERISATION OF *DICHELOBACTER NODOSUS* FROM FOOTROT INFECTED SHEEP IN MALAYSIA

Sheikh Omar Abdul Rahman, Zunita Zakaria, Abdul Rahim Mutalib and Son Radu

Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Keywords: footrot, *Dichelobacter nodosus*, sheep, Malaysia.

Introduction

Dichelobacter nodosus is the causative agent of severe footrot, a contagious debilitating disease which affects ruminants particularly sheep and goats. The disease is characterised by separation of the horn of the hoof from the underlying soft tissues resulting in lameness, loss of body condition and reduced wool production (Rood et al. 1996). The first case of footrot in Malaysia was detected in early 1994 at Institut Haiwan Kluang farm and the disease is now known to be present in several other farms. Information on the etiological agent of the disease is important to understand the situation in Malaysia enabling of suitable measures to control and if possible to eradicate footrot. This study involved isolation, identification and characterisation of *D. nodosus* isolates.

Materials and Methods

D. nodosus isolates were obtained from sheep suffering from footrot from three farms in Johor, Kedah and Terengganu. Lesion materials were inoculated onto 4% hoof agar (HA) plates (Egerton and Roberts, 1971) and incubated anaerobically at 37°C for 3-4 days. Suspected colonies were purified on 4% HA. Serogrouping was done by slide agglutination test as described by Claxton et al. (1983) and the serotype was determined by the microtitre plate agglutination test. Confirmation of field isolates was done by PCR using specific primers. The elastase and gelatin gel test were applied to isolates in order to determine the virulence. Isolates inoculated on elastin agar were observed for their elastase activity on 4, 7, 11, 17, 21 and 24 days post-incubation (p.i.). Isolates were recorded positive on a particular day p.i. if a zone of elastin clearance was observed around the inoculum. Proteases were recorded as either thermostable or thermolabile depending on whether a zone of gelatin clearance was observed after 16 minutes of prior incubation of a broth culture at 68°C. The pulsed field electrophoresis (PFGE) technique (Chu et al. 1986) was used for genomic typing of *D. nodosus* isolates.

Results and Discussion

D. nodosus was isolated from 12 of 30 infected sheep with footrot lesion score 2 from three separate farms. They were designated Mal 1 to Mal 12. Diagnosis was done by Gram-staining while laboratory confirmation was accomplished by PCR technique. All 12 isolates reacted positively in the PCR method by producing a single product of 783 base pair. All isolates, although obtained from different locations, were found to be of serogroup B (10 isolates were B2 serotype, 2 isolates were B1 serotype). The occurrence of a single serogroup in Malaysia out of nine serogroups currently recognised for *D. nodosus,* is rather unique. In other countries such as Australia , New Zealand and Britain, at least three serogroups were present in their flocks (Claxton et al. 1983). Results of serotyping later showed that the isolates were quite distinct from the four B group prototype strains. Although the isolates were designated to a certain serotype within the serogroup, they exhibited low agglutinating titres for the prototype antisera. These results indicated that the isolates possessed only a few shared antigens with the prototype strains, thus possessing less agglutinating epitopes with prototypes antisera. The assessment of the virulence of each isolate was done by two standard conventional methods, namely the elastase and gelatin-gel tests. Both tests showed a high level of agreement and repeatability. Therefore either of the tests is suitable for use in determining the virulence of isolates. Generally the isolates exhibited a variation in the laboratory characteristics although they had been isolated from lesions with a similar score. Mal 1, Mal 8 and Mal 12 isolates which appeared to have the ability to cause virulent footrot with their positive elastase and thermostable protease characteristics, caused only a mild form of the disease producing lesions with score 2. This manifestation is probably due to the constant topical treatment regimen adhered to by the farm management and the result of the vaccination programme causing the bacteria not to fully express its virulence. PFGE analysis was used to compare chromosomal DNA restriction patterns in order to differentiate between isolates. It was found that *D. nodosus* isolates could readily be analysed by this technique. Significant patterns were generated by three GC-rich enzymes (*Apa*I, *Sfi*I and *Sma*I). A considerable genetic diversity was found among the *D. nodosus* isolates (eight genome types form 12 isolates). It is of interest that although opportunities for cross infection between flocks were high, no particular genome type was predominant among the flocks studied.

Conclusions

D. nodosus isolates in Malaysia which belong to a single serogroup (serogroup B), are variable in their capability to cause different degrees of footrot. The isolates also demonstrated multiple genotypes even though they were obtained from the same flock. It is highly probable that the isolates were from diverse sources. The source of this heterogeneity could not be defined in this study, although it is most likely the result of both multiple environmental exposure and genetic drift within the population.

References

Chu, G., Vollrath, D., Davis, R.W. 1986. Separation of large DNA molecules by contour clamped homogenous elactric field. *Sci. 234*, 1582-1585.

Claxton, P.D., Ribeiro, L.A. and Egerton, J.R. 1983. Classification of *Bacteroides nodosus* by agglutination tests. *Aust. Vet. J. 60*(11), 331-334.

Egerton, J.R. and Roberts, D.S. 1971. Vaccination against ovine footrot. *J. Comp. Path. 81*, 179-185.

Rood, J.I., Howarth, P.A., Haring, V., Billington, S.J., Yong, W.K., Liu, D., Palmer, M.A., Pitman, D.R., Links, I., Stewart, D.J. and Vaughan, J.I. 1996. Comparison of gene probe and conventional methods for the differentiation of ovine footrot isolates of *D. nodosus*. *Vet. Micro.* 52: 127-141.

Supported by IRPA Grant 01-02-04-0211