PULSED FIELD GEL ELECTROPHORESIS ANALYSIS OF *SERPULINA HYODYSENTERIAE* ISOLATES

R.F. Atyeo and D.J. Hampson
Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia 6150

Introduction

Molecular typing techniques have been used to determine the extent of strain variation in *Serpulina hyodysenteriae*, and have been useful in studying the molecular epidemiology of swine dysentery (SD). Techniques used have included multilocus enzyme electrophoresis (MEE) (6), DNA restriction endonuclease analysis (REA) (3), restriction fragment length polymorphism (RFLP) analysis (5), and random amplification of polymorphic DNA (RAPD) analysis (4). In the current study, pulsed field gel electrophoresis (PFGE) was developed as a simple and reliable alternative method for subspecific differentiation of the species.

Material and methods

Forty isolates of *S. hyodysenteriae* from Australian herds, assigned to 12 different electrophoretic types (ETs) in MEE, 21 REA types and six serogroups were used initially (6). All were confirmed as *S. hyodysenteriae* using a specific PCR (2), and by their phenotypic properties and their MEE profile (6). Another 29 strains isolated from pigs on four farms were examined in order to determine the potential of PFGE as an epidemiological typing tool.

The PFGE protocol was as previously reported for *S. pilosicoli* (1), but with minor modifications. Gels were loaded onto a contour-clamped homogenous electric field-DR 11 system (BioRad Laboratories, USA), and were subjected to electrophoresis at 180V for 18h at 14^{0}C, with an initial switch time of one s and a final switch time of 50 s, and a linear ramp. Patterns were initially categorised by eye, and the predominant PFGE types were then analysed by scanning photographs (Ofoto 2.0) into the Molecular Analyst programme, version 1.0. This programme created a dendrogram from a matrix of band matching coefficients (Fuzzy Logic) by the UPMGA clustering fusion strategy.

Results and discussion

*Mlu*I was the most consistent and efficient enzyme for cleaving *S. hyodysenteriae* DNA, on average generating 8-10 DNA bands per strain. More problems were experienced with wide banding patterns, DNA shearing and DNA degradation than had previously been found with *S. pilosicoli*(1).

The first 40 reference isolates were divided into 23 PFGE types, whilst the 29 field isolates were divided into seven PFGE types. From these results it was clear that PFGE analysis was more able to discriminate strains than was MEE. Overall the results of PFGE correlated most closely with the REA results, whilst the results of serogrouping did not correlate well with any of the methods. PFGE analysis was particularly valuable as it much easier to interpret than REA, as less bands were obtained and these were spaced further apart on the gel.

Little variation was seen amongst the strains of *S. hyodysenteriae* isolated from individual herds over time. At most, only two PFGE types of *S. hyodysenteriae* were found in an individual piggery. These results confirm previous findings using REA, where it was shown that there are a number of common or predominant strains of *S. hyodysenteriae* present in piggeries in the different states of Australia (3). A similar situation has been described amongst strains of *S. hyodysenteriae* affecting herds in Canada, which were typed by using RAPD analysis (4).

The percentage similarity of the *S. hyodysenteriae* isolates by PFGE analysis ranged from approximately 53% to 100%. These results suggest that the *S. hyodysenteriae* population studied was not particularly genetically diverse.

Acknowledgements

This study was funded by the Australian Pig Research and Development Corporation.

References

1 Atyeo, R. F, Oxberry, S. L. and Hampson, D. J. (1996) Pulsed field gel electrophoresis for subspecific differentiation of *Serpulina pilosicoli* (formerly '*Anguillina coli*'). *FEMS Microbiology Letters*, **141**, 77-81.

2 Atyeo, R. F., Oxberry, S. L., Combs, B. G. and Hampson, D. J. (1998) Development and evaluation of polymerase chain reaction tests as an aid to the diagnosis of swine dysentery and intestinal spirochaetosis. *Letters in Applied Microbiology*, in press

3 Combs, B. G., Hampson, D. J. and Harders, S. J. (1992) Typing of Australian isolates of *Treponema hyodysenteriae* by serology and by DNA restriction endonuclease analysis. *Veterinary Microbiology*, **31**, 273-285

4 Dugourd, D., Jacques, M., Bigras-Poulin, M. and Harel, J. (1996) Characterisation of *Serpulina hyodysenteriae* isolates of serotypes 8 and 9 by random amplification of polymorphic DNA analysis. *Veterinary Microbiology*, **48**, 305-314

5 Duhamel, G. E., Ramanathan, M., Bernard, R. J., Newman, M. C., Erickson, E. D. (1992) Application of restriction fragment length polymorphism typing to epidemiological tracing of *Serpulina hyodysenteriae*. *Proceedings of the 12th International Pig Veterinary Society Congress*, The Hague, Netherlands. p 276

6 Lee, J. I., Hampson, D. J., Combs, B. G. and Lymbery, A. J. (1993) Genetic relationships between isolates of *Serpulina* (*Treponema*) *hyodysenteriae*, and comparison of methods for their subspecific differentiation. *Veterinary Microbiology*, **34**, 35-46