



THE PREVALENCE AND MOLECULAR EPIDEMIOLOGY OF *SERPULINA PILOSICOLI* IN PIGS IN THE EASTERN HIGHLANDS OF PAPUA NEW GUINEA

¹A.S.J. Mikosza, ¹D.J. Trott, ²B.G. Combs, ¹S.L. Oxberry and ¹D.J. Hampson

¹Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia, 6150 ²PNG Institute of Medical Research, Goroka, Eastern Highlands Province, Papua New Guinea.

Introduction

Serpulina pilosicoli is the causative agent of intestinal spirochaetosis (IS), a diarrhoeic condition characterised by the end-on attachment of the spirochaetes to the epithelium of the large intestine. A previous study of porcine isolates of *S. pilosicoli* using pulsed field gel electrophoresis (PFGE) indicated a high genetic diversity of isolates, although these were collected from a range of geographical locations (1). Although a number of epidemiological studies of *S. pilosicoli* in pigs have been made in western countries (2, 3), none have been performed in other regions of the world. An epidemiological study was carried out in the Eastern Highlands Province of Papua New Guinea, in order to determine the epidemiology of *S. pilosicoli* in villagers and domestic animals. The study centered upon European Large White-Landrace pigs in an intensive commercial piggery, and an indigenous species of pig, *Sus scrofa papuensis*, which were found in nearby villages, and reared under non-intensive conditions.

Material and Methods

Faecal samples were obtained from 50 commercial pigs and 126 indigenous pigs from four villages. The samples were immediately plated onto anaerobic selective media containing the antibiotics spectinomycin (400 µg/mL), colistin (25 µg/mL) and vancomycin (25 µg/mL) (4). Spirochaete colonies were identified as haemolytic streaks on blood agar and phase contrast microscopy. All isolates were transported to Murdoch University where they were re-cultured and further examined by molecular techniques. A species specific polymerase chain reaction (PCR) (5) and multilocus enzyme electrophoresis (MEE) (6) were used to confirm the isolates as *S. pilosicoli*, while MEE and PFGE (1) were used to analyse the sub-specific genetic variation of the isolates.

Results and Discussion

A high rate of infection by intestinal spirochaetes (21 of 50 = 42%, consisting of 6 of 10 weaner pigs, 14 of 30 grower pigs, and 1 of 10 finisher pigs) was found in the commercial piggery. In stark contrast to this, there were no intestinal spirochaetes isolated from any indigenous pig. These pigs had every opportunity to be infected, as they scavenged around the villages and surrounding bush where there was free access to human faeces, which was known to be contaminated with *S. pilosicoli* (7). As well, in a related study, village dogs were found to be infected by human strains of *S. pilosicoli* (8). It has been previously shown that European pigs can be infected by human strains of *S. pilosicoli* (9), and it may be that colonisation may be influenced by genetic differences between the indigenous *Sus scrofa papuensis*, and the European Large White-Landrace pigs of the commercial piggery. Diet may have been an influence, as a study on the related spirochaete, *Serpulina hyodysenteriae*, indicated that pigs on standard commercial diets were more susceptible to infection (10), and this may also apply to *S. pilosicoli*. The environment may also have been an influence, with the village pigs being raised in a low population density outdoor environment, as opposed to the intensive environment of the commercial pigs. Previous studies in villages in the same region have shown a low carriage rate of *Streptococcus suis* type 2 (11) and rotavirus (12) in village pigs, which was attributed to the non-intensive husbandry procedures adopted in the villages.

When molecular techniques were applied, fourteen of the 21 spirochaete isolates (66%) were determined to be *S. pilosicoli* by PCR and MEE, two isolates (9.5%) were shown to be *Serpulina intermedia* by MEE, and five isolates were lost during transport or subsequent contamination. MEE divided the 14 *S. pilosicoli* isolates into eight electrophoretic types (ETs), while PFGE further divided these isolates into 12 PFGE types, indicating a very high *S. pilosicoli* strain diversity in the commercial piggery.

The commercial pigs appeared ill-thrifty, had a poor growth rate, and mucoid diarrhoea was observed on the floors of the pens. However, no significant association was demonstrated between colonisation of commercial pigs with intestinal spirochetes, and faecal consistency. The large number of strains of *S. pilosicoli* found in the commercial pigs may explain the tendency for IS to persist in a piggery, and for the disease to recur in recovered animals.

References

- 1 Atyeo R. F., Oxberry S. L., Hampson D. J., (1996) Pulsed-field gel electrophoresis for sub-specific differentiation of *Serpulina pilosicoli* (formerly "*Anguillina coli*"). *FEMS Microbiology Letters*, **141**, 77-81.
- 2 Lee, J. I., (1994) Characterisation of intestinal spirochaetes from pigs, dogs and man, Ph.D. Thesis, Murdoch University, Perth, pp. 260.
- 3 Fellström, C., and Gunnarsson, A., (1995) Phenotypical characterisation of intestinal spirochaetes isolated from pigs. *Research in Veterinary Science*, **59**, 1-4.
- 4 Jenkinson, S. R., and Wingar, C. R., (1981) Selective medium for the isolation of *Treponema hyodysenteriae*, *The Veterinary Record*, **109**, 384-385.
- 5 Park, N. Y., Chung, C. Y., McLaren, A. J., Atyeo, R. F., and Hampson, D. J., (1995) Polymerase chain reaction for identification of human and porcine spirochaetes recovered from cases of intestinal spirochaetosis, *FEMS Microbiology Letters*, **125**, 225-230.
- 6 Lee, J. I., Hampson, D. J., Lymbery, A. J., and Harders, S. J., (1993) The porcine intestinal spirochaetes: identification of new genetic groups, *Veterinary Microbiology*, **34**, 273-285.
- 7 Trott, D. J., Combs, B. G., Mikosza, A. S. J., Oxberry, S. L., Robertson, I. D., Passey, M., Taimre, J., Sehuko, R., Alpers, M. P., and Hampson, D. J., (1997) The prevalence of *Serpulina pilosicoli* in humans and domestic animals living in the Eastern Highlands of Papua New Guinea, *Epidemiology and Infection*, **119**, 369-379.
- 8 Trott, D. J., Mikosza, A. S. J., Combs, B. G., Oxberry, S. L., Hampson, D. J., (1998) Population genetic analysis of *Serpulina pilosicoli* and its molecular epidemiology in villages in the Eastern Highlands of Papua New Guinea, *International Journal of Systematic Bacteriology*, In Press.
- 9 Trott, D. J., Huxtable, C. R., and Hampson, D. J., (1996) Experimental infection of newly weaned pigs using human and porcine strains of *Serpulina pilosicoli*, *Infection and Immunity*, **64**, 4648-4654.
- 10 Siba, P. M., Pethick, D. W., and Hampson, D. J., (1996) Pigs experimentally infected with *Serpulina hyodysenteriae* can be protected from developing swine dysentery by feeding them a highly digestible diet, *Epidemiology and Infection*, **116**, 207-216.
- 11 Paterson, R. A., Robertson, I. D., Sanders, R. C., Siba, P. M., Clegg, A., and Hampson, D. J., (1993) The carriage of *Streptococcus suis* type 2 by pigs in Papua New Guinea, *Epidemiology and Infection*, **110**, 71-78.
- 12 Alpers, D., Sanders, R. C., and Hampson, D. J., (1991) Rotavirus excretion by village pigs in Papua New Guinea, *Australian Veterinary Journal*, **68**, 65-67.