DISTRIBUTION AND STRAIN TYPING OF *BRACHYSPIRA PILOSICOLI* IN TWO WESTERN AUSTRALIAN PIGGERIES

S.L. Oxberry and D.J. Hampson
Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch 6150 WA, Australia

Introduction

The intestinal spirochaete *Brachyspira pilosicoli* causes intestinal spirochaetosis (IS), a diarrhoeal disease first recognised in 1980 (1). This condition results in production losses due to reduced growth rates and poor feed conversion. The purpose of this study was to investigate the distribution and strain diversity of *B. pilosicoli* on two piggeries in Western Australia

Materials and Methods

Two piggeries were selected for study. Piggery A was a >2000 sow unit that had had problems with poor growth rates in the grower shed associated with *B. pilosicoli* infection. Initially 45 samples from problem areas at the farm were examined, then a cross-sectional study was conducted which examined 255 faecal samples taken from healthy animals, including growers, finishers and boars. A cohort study following four litters of piglets (37 pigs) from 3 weeks of age until slaughter at 6 months then was conducted, with 220 faecal swabs collected at 8 samplings.

Piggery B was located on a research station, and it obtained stock piglets from piggery A. A cross sectional study was carried out on faecal samples from 72 grower and finisher pigs (discussed in (2)), and a cohort study examining faecal samples from 39 animals from five litters also was conducted. Samples were taken every fortnight from 3 weeks of age until slaughter at 6 months of age. A total of 362 samples were collected.

All faecal samples were plated on selective Trypticase Soy blood agar plates which were incubated anaerobically at 37°C for up to 14 days. Surface growth on the plates was harvested and subjected to an *B. pilosicoli* -specific polymerase chain reaction amplifying a portion of the 16S rRNA gene (3).

Isolates obtained were subjected to multilocus enzyme electrophoresis (MLEE) (4), and pulsed field gel electrophoresis (PFGE) (5) to obtain information on strain diversity. An isolate previously obtained from an effluent lagoon and four isolates from ducks living on the lagoon at piggery B (2) also were subjected to MLEE.

Results

Cross-sectional study on piggery A: B. pilosicoli was isolated from five (11.1%) of the 45 samples that were taken from animals with clinical signs of IS and from four (1.6%) samples obtained from the 255 healthy pigs sampled during the cross-sectional study. *B. pilosicoli* DNA was amplified from 10 (22.2%) of the 45 clinically ill pigs and from 5 (2.0%) of the 255 healthy animals. The nine isolates all belong to three electrophoretic types (ETs) in MLEE and three PFGE types.

Cohort study on piggery A: Only one isolate of *B. pilosicoli* was cultured from a six month old pig. PCR was not performed on any of the samples. MLEE and PFGE analysis on this sample showed that it belonged to the same ET and PFGE type as six of the isolates obtained in the cross-sectional study from this piggery.

Cross sectional study on piggery B: Four isolates of *B. pilosicoli* were cultured and these fell into four distinct electrophoretic types. Isolate WPP 22 belonged to the same ET as a single isolate of *B. pilosicoli* obtained in an earlier study from an effluent lagoon on the same piggery (2). The four isolates of *B. pilosicoli* obtained from ducks on this farm were genetically distinct from each other and from those from the effluent water and pigs.

Cohort study on piggery B: Fifteen of the 39 pigs (38.5%) sampled were infected with *B. pilosicoli* at some point. One pig gave a positive PCR result twice over the six month sampling period and three isolate (WPP 17, WPP 18 and WPP 19) of *B. pilosicoli* were obtained from another animal at 13, 19 and 21 weeks of age. *B. pilosicoli* DNA was detected in pigs at a variety of sampling times. The six isolates obtained were grouped into three ETs and four PFGE types, one of which also contained seven isolates obtained from piggery A. Isolates WPP 18 and WPP 19 obtained at weeks 19 and 21 were distinct from WPP 17 obtained from the same pig at week 13.

Discussion

PCR from the primary plates was more sensitive for detection of *B. pilosicoli* than was culture, with up to two thirds of the samples yielding *B. pilosicoli* DNA having no organisms isolated. Generally prevalence rates were high in pigs with diarrhoea (22.4%), but were low overall in the cross-sectional surveys of healthy pigs (2% in piggery A and 12% in piggery B). This distribution suggests that *B. pilosicoli* was involved in the aetiology of diarrhoea. The cohort study on piggery A suggested that *B. pilosicoli* infection was rare, despite its association with certain groups of pigs with diarrhoea in the grower shed. The cohort study on piggery B revealed a higher prevalence, and indeed nearly 40% of all pigs excreted *B. pilosicoli* at some point (based on PCR results). Infection occurred in various age groups of pigs. The duration of infection with a single strain appeared to be at least 2 weeks. This is consistent with the finding of other researchers (6). Several distinct strains of *B. pilosicoli* were present on both piggeries, with one strain being common to both (ET2). This strain may have been introduced into piggery B in piglets obtained from piggery A. This same strain was the most common strain on piggery A, and was associated with diarrhoea in growers. The isolate from efflent water on piggery B was the same as one recovered from a pig on piggery B, whilst four isolates from wild ducks on the piggery were all distinct from each other and from strains recovered from the pigs. These findings emphasise the complex molecular epidemiology of *B. pilosicoli* infections on piggeries, and suggests that some strains may be more associated with disease in pigs than others.

Acknowledgements

This study was funded by a grant from the Australian Pig Research and Development Corporation. Thanks are due to RJ Buddle and JM Accioly for assistance with the collection of faecal specimens.

References

1. Taylor, DJ, Simmons, JR, and Laird, HM (1980). Production of diarrhoea and dysentery in pigs by feeding pure cultures of a spirochaete differing from *Treponema hyodysenteriae*. *Veterinary Record*, **106**: 326-332.
2. Oxberry, SL, and Hampson, DJ (2000). Isolation of *Brachyspira pilosicoli* from environmental and other sources on a piggery. *16th IPVS congress*, Melbourne, Australia.
3. Park, NY, Chung, CY, McLaren, AJ, Atyeo, AJ, and Hampson, DJ (1995) Polymerase chain reaction for identification of human and porcine spirochaetes recovered from cases of intestinal spirochaetosis. *FEMS Microbiology Letters*, **125**: 225-230.
4. Lee JI, Hampson DJ, Lymbery, AJ, Harders, SJ (1993) The porcine intestinal spirochaetes: identification of new genetic groups. *Veterinary Microbiology*, **34**: 273-285.
5. Atyeo RF, Oxberry SL, & Hampson DJ (1996) Pulsed-field gel electrophoresis for sub-specific differentiation of *Serpulina pilosicoli* (formally *"Anguillina coli"*). *FEMS Microbiology Letters*, **141**, 77-81.
6. Duhamel, GE (1998) Colonic spirochaetosis caused by *Serpulina pilosicoli. Large Animal Practice,* **19**: 14-22.