POLYMERASE CHAIN REACTION TARGETTING THE *NOX* GENE FOR IDENTIFICATION OF *SERPULINA INTERMEDIA* IN PIGS

[1]R.F. Atyeo, [1]D. Suriyaarachchi, [2]T.B. Stanton, [2]N.S. Jensen, and [1]D.J. Hampson

[1]Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA, Australia, [2]National Animal Disease Center, Ames, Iowa, USA

Introduction

Serpulina intermedia (formerly "*S. intemedius*") is a recently named species of weakly ß-haemolytic, indole positive anaerobic intestinal spirochaete (7). In diagnostic laboratories it can be easily confused with the strongly ß-haemolytic, indole positive *S. hyodysenteriae* (the agent of swine dysentery), or one of several other weakly ß-haemolytic non-pathogenic *Serpulina* species. *S. intermedia* is considered to be a pathogen of poultry (5), however evidence for its pathogenic potential in pigs remains equivocal (3). Strains of what appear to be *S. intermedia* have been isolated from pigs with diarrhoea in Poland (1) and Sweden (2), but in experimental studies infection of conventional pigs with *S. intermedia* type strain PWS/A did not result in disease (4). The purpose of this study was to develop a polymerase chain reaction (PCR) test for the identification of *S. intermedia* strains using sequence information derived from the NADH oxidase (*nox*) gene of the spirochaete. NADH oxidase has been detected in every *Serpulina* strain tested and thus may be an identifying trait for the genus (7).

Materials and methods

Sequencing of the *nox* genes was carried out on PCR-amplified DNA from *S. hyodysenteriae* strains B78^T, B169 and R1, *S. innocens* strains B256^T, 4/71 and P280/1, porcine *S. intermedia* strains PWS/A^T, 2818.5 and 4482, *S. murdochii* strains 56-150^T and 155-20, *S. pilosicoli* strains P43/6/78^T, HRM7, and WesB, *S. alvinipulli* strain C1^T (8), the unclassified chicken spirochaete 42167, and *Brachyspira aalborgi* strain ATCC 43994. The sequences were aligned and primers specific for *S. intermedia* were identified.

The species-specific PCR was carried out using a hot start protocol, with the bottom phase consisting of 5% buffer, 10nmoles of nucleotides, 4mM magnesium chloride, and 3.3pmoles of each primer. A 20µl layer of wax was used to separate the phases. The top phase consisted of 5% buffer, 0.55 units of thermostable DNA polymerase enzyme and 100ng of target DNA. Equal portions of sterile milli-Q filtered water was added to each phase to bring the total volume to 25µl. Thermal cycling consisted of an initial denaturation period of one min at 95°C, followed by 30 cycles of 30s at 95°C, one min at 45°C, two min at 72°C, and a final annealing and extension cycle of one min at 45°C and 10 min at 72°C. The PCR test was optimised using 79 isolates; 21 isolates of *S. hyodysenteriae*, 21 isolates of *S. pilosicoli*, 10 isolates of *S. innocens*, 5 isolates of *S. murdochii*, 10 porcine and 10 avian isolates of *S. intermedia*, *Brachyspira aalborgi*, and *S. alvinipulli* strain C1. The detection of PCR products was carried out by conventional horizontal gel electrophoresis, soaking in ethidium bromide (0.5µg/ml solution) for 30 min, and visualising by uv transillumination.

Results and discussion

Differences in the sequence of the *nox* gene in the various species were identified, and potentially species-specific primer sites were identified. The primer sequences used for the detection of *S. intermedia* were: forward primer SINTF1 - GTC CTG AAA GCT TAA AAA, reverse primer SINTR1 - CTA ATA AAC GTC CAG TAT. This PCR always produced a single band product of the expected 1004 bp size. The PCR conditions were optimised so that the DNAs from all the *S. intermedia* strains from pigs was amplified, whilst no product was generated from other *Serpulina* spp.. Unfortunately under these conditions DNA from only four of the ten isolates of *S. intermedia* from chickens was amplified.

The PCR test described in this study was robust, and was useful in that it could be used to rapidly identify porcine strains of *S. intermedia*. The reason why only some chicken isolates of *S. intermedia* were amplified is uncertain, since all were indole positive and had been classified as *S. intermedia* by multilocus enzyme electrophoresis (McLaren *et al*, 1997). DNA-DNA reassociation studies are required to clarify the species identity of the PCR-negative subgroup of indole positive spirochaetes. It would also be useful to examine and compare the *nox* gene sequences of the strains that were positive and negative in the PCR, to help determine why the negative strains failed to amplify. Currently there is a requirement for a better definition of the species *S. intermedia* and an understanding of its genetic and phenotypic diversity. The ability to confidently identify strains of *S. intermedia* is important for assessing virulence of strains of the species.

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