Proceedings

The 13th International Pig Veterinary Society Congress
26-30 June 1994, Bangkok, Thailand.

Correction page 505

5 % pentobarbital should read 0.5 % pentobarbital

line 2 in Abstract
line 10 in Materials and methods
line 1 in Table 1
line 2 in Table 2
line 7 in Discussion

Organized by Chulalongkorn University Faculty of Veterinary Science
Edited by Prachak Poomvises & Pringsri Ingkaninun

The use of polymerase chain reaction for the identification of *Serpulina hyodysenteriae*

B.G. Combs, R.F. Atyeo and D.J. Hampson*

School of Veterinary Studies, Murdoch University, Murdoch, 6150, Western Australia.

Introduction

The isolation and identification of *Serpulina hyodysenteriae* is considered necessary to obtain a definitive diagnosis of swine dysentery (1). Identification has traditionally been carried out using phenotypic tests, although recently DNA hybridisation tests have been reported as an alternative (2). The usefulness of these and other tests relies on their ability to distinguish between *S. hyodysenteriae* and other spirochaetes that are frequently isolated from the porcine colon (3). Identification of pathogenic bacteria has been successfully carried out using the polymerase chain reaction (PRC) (4), and in the current study this method was investigated as a means of identifying *S. hyodysenteriae*.

Materials and methods

The DNA sequence chosen for use in the development of the PCR originated from a 1700 base pair probe sequence previously shown to be useful for identifying *S. hyodysenteriae* (5). This 1700 bp DNA fragment was subcloned into plasmid pUC19 and transformed into *E. coli* strain JM109. The recombinant insert was partially sequenced with the dideoxy reaction method using a M13 reverse primer combined with cycle sequencing. The sequence reaction was analysed using an automated sequencing system supplied by Applied Biosystems. Sequence information for 323 bases was analysed for suitable primer sites using the computer program "Primer Designer" (Scientific and Educational Software, USA). Two primers, BGC2 and BGC3, each of 20 bases, were chosen: these would theoretically produce a product 288 base pairs long. These primers were used in a PCR reaction volume of 25 µl containing 1.5 mM $MgCl_2$, 1 unit of Tth plus (DNA polymerase, Biotech International), 200 uM of nucleotides dATP, dTTP, dCTP and dGTP, 25 pmol of primers and 100 ng of spirochaetal DNA.

The PCR was performed in a MJ Scientific thermocycler using a protocol involving an initial cycle of 94°C for 3 min, then 61°C for 20 sec and 72°C for 20 sec. The next 34 cycles were a repeat of this cycle except that the 94°C step was for only 20 sec. The cycling concluded with a 72°C step for 10 minutes. The PCR reactions were examined for DNA product by agarose gel electrophoresis using a 1.5% agarose gel, and staining with ethidium bromide. The specificity of the test was examined by using DNA from 30 isolates of *S. hyodysenteriae* and 30 non-*S. hyodysenteriae* spirochaete isolates. The *S. hyodysenteriae* isolates were identified by their strong beta haemolysis, positive indole production and characteristic API-ZYM profile. The non-*S. hyodysenteriae* spirochaetes were all weakly beta haemolytic, although 10 were positive for indole production and had an *S.hyodysenteriae* API-ZYM profile. Phenotypic and genetic analysis of the non-*S. hyodysenteriae* spirochaetes identified them as belonging to four separate genetic groups: *Serpulina innocens*, *"Serpulina murdochii"*, *"Serpulina intermedius"* and *"Anguillina coli"* (6, 7). These reaction conditions were also used on serial dilutions of *S. hyodysenteriae* DNA to determine the lower limit of detection for the test.

Results

All 30 *S. hyodysenteriae* isolates produced an amplified DNA product, although with some isolates the product was larger than expected. At a primer annealing temperature of 61°C none of the 30 non-*S. hyodysenteriae* isolates produced an observed product, although when the temperature was lowered a product did become visible. Using these reaction conditions the lowest concentration of DNA that the test could detect was 10 ng.

Discussion

PCR has been shown to be capable of being used for identifying pathogenic bacteria, either with purified DNA, bacterial cell preparations or clinical specimens. In this study, under the conditions specified, the PCR test was able to identify all 30 isolates of *S. hyodysenteriae*. These were from different countries, and represented different serogroups and genetic groups (based on multilocus enzyme electrophoresis). The specificity of the test was also good, as no amplified product was seen when DNAs from 30 non-*S. hyodysenteriae* porcine spirochaetes were used. These isolates were chosen as they represent a cross-section of porcine spirochaetes that might be isolated from pigs, some of which have caused problems in the past for tests developed for the identification of *S. hyodysenteriae* (2). The test was able to detect a minimum of 10 ng of *S. hyodysenteriae* DNA, but this is not as sensitive as PCR tests for some other bacteria (4). In the future we hope to investigate the use of other primers in a PCR test that will enable the detection of lower concentrations of *S. hyodysenteriae* DNA. This may be of benefit in detecting asymptomatic carrier pigs shedding *S. hyodysenteriae*.

References

1. Harris, D.L. and Lysons, R.J. (1992). Chapter 49. In "Diseases of Swine" ed. Leman, A.D., Straw, B.E., Mengeling, W.L., D'Allaire, S. and Taylor, D.J., Iowa State University Press, Ames, Iowa.

2. Jensen, N.S., Casey, T.A. and Stanton, T.B. (1990) J. Clin. Microbiol. 28 (12) : 2717-2721.

3. Joens, L.A., Songer, J.G., Harris, D.L. and Kinyon, J.M. (1980) Vet. Rec. 106 : 245-246.

4. Woodward, M.J., Carroll, P.J. and Wray, C. (1992) Vet. Microbiol. 31 : 251-261.

5. Combs, B.G. and Hampson D.J. (1992) Proc. 12th Int. Pig. Vet. Soc. Cong. The Hague. 275.

6. Lee, J.I., Hampson, D.J., Lymbery, A.J., and Harders, S.J. (1993) Vet. Microbiol. 34 : 273-285.

7. Lee, J.I. and Hampson, D.J. (1994) J. Med. Microbiol. (in press).