DIAGNOSIS OF SWINE DYSENTERY AND INTESTINAL SPIROCHAETOSIS BY THE USE OF POLYMERASE CHAIN REACTION TESTS ON FAECAL SAMPLES

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The polymerase chain reaction (PCR) is a technique based on amplification of specific DNA sequences, the products of which may be detected using electrophoresis (Saiki et al., 1985). The DNA sequences from Serpulina hyodysenteriae, the causative agent of swine dysentery (SD) (Combs and Hampson, 1992), and from the spirochaete previously called “Anguillina coli” (Lee et al., 1993), which is associated with intestinal spirochaetosis (IS) (Park et al., 1995), were used to design PCR tests, and these were applied to the detection of these bacteria in pigs.

The PCRs were optimised by applying the tests to purified DNA extracted from 49 strains of S. hyodysenteriae, 12 strains of the spirochaete associated with IS, and 46 strains from other closely related genetic groups, such as S. innocens, S. intermedius, S. murdochii and Brachyspira aalborgi, all of which had been previously differentiated using the technique of multilocus enzyme electrophoresis (Lee et al., 1993; Lee and Hampson, 1994). These included strains from Australia, the UK, the USA, and Canada. These were isolated mainly from pigs, but strains from humans, chickens, rheas, and an isolate from a swan were included. Both PCRs were specific for the organism they were designed to detect, and an internal oligonucleotide probe was used to confirm the identity of the S. hyodysenteriae DNA product. Variations on the PCR protocols to increase their sensitivity, including the use of hot starts and nested reactions, were evaluated.

The treatment of faeces prior to PCR is an important part of clinical diagnosis, because of the large number of PCR inhibitors which are present in faeces (Newton and Graham, 1994). A number of different regimes for preparing the faeces were examined, including the use of immunomagnetic separation of spirochaetes, commercial DNA extraction kits, Instagene, phenol/chloroform DNA extraction, diatomaceous earth DNA extraction, and culture resuspension prior to diatomaceous earth extraction (CRDEX).

In the latter technique the total growth on blood agar plates from samples which had been cultured was collected and the DNA harvested. The CRDEX technique was the best method of treatment, since $10^2$-$10^4$ spirochaetes could be detected in 0.2 g of faeces. All other techniques could only detect $\leq 10^6$ spirochaetes in 0.2 g of faeces. The use of CRDEX would be likely to correctly diagnose pigs with clinical SD or IS, but might not detect carrier pigs, which would probably be shedding fewer organisms than this (Kinyon et al., 1977).

Combinations of CRDEX and PCR tests were applied to 22 porcine clinical faecal samples, and to the faeces of seven experimentally infected pigs. The tests successfully detected the two spirochaetes respectively in all samples from which they could be cultured and isolated, and from four samples from which spirochaetes could not be isolated. The CRDEX technique reduced the time required for diagnosis from three weeks to less than ten days. The use of sequential PCRs on single DNA extracts is becoming an important tool for the rapid diagnosis of both SD and IS.

References


