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Comparison of culture and biochemical tests with PCR for detection of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*

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Abstract

Traditional culture and biochemical tests (CBT) were compared with PCR for sensitivity and detection of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* in seeded faeces and clinical samples from diarrhoeic pigs. A duplex PCR system was developed based on primers detecting the *tly*A-gene of *B. hyodysenteriae* and the 16S rRNA-gene of *B. pilosicoli*. Sensitivities for the PCR system were determined on seeded faeces, using DNA that had been recovered from primary cultures or extracted directly from faeces. Compared to CBT, PCR applied to DNA extracted directly from faeces lowered the sensitivity by a factor of 1000 to 10,000. *B. hyodysenteriae* and *B. pilosicoli* detection was compared for CBT and PCR using 200 clinical samples. CBT detected more *B. hyodysenteriae* isolates in the clinical samples than PCR, but fewer *B. pilosicoli* positive samples. An atypical strongly haemolytic isolate was detected only by CBT.

Keywords: Atypical isolate; *Brachyspira*; Culture; Faecal DNA extraction; PCR; Sensitivity

1. Introduction

Swine dysentery (SD) and porcine intestinal spirochaetosis (PIS) are pig diseases caused by *Brachyspira* spp. Swine dysentery is caused by *Brachyspira hyodysenteriae* (Taylor and Alexander, 1971) and causes a severe mucohaemorrhagic diarrhoea. Porcine intestinal spirochaetosis, also called spirochaetal diarrhoea or colonic spirochaetosis, is caused by *Brachyspira pilosicoli* and characterised by growth loss and mild, greyish diarrhoea (Taylor et al., 1980). Currently, five *Brachyspira* species have been described in pigs but only two of these are considered pathogenic, i.e. *B. hyodysenteriae* and *B. pilosicoli* (Harris et al., 1972; Hudson et al., 1976; Kinyon and Harris, 1979; Thomson et al., 1997). Pigs suffering from SD and PIS shed 10^8–10^{10} bacterial cells per gram faeces in the acute phase of the disease. Pigs not treated with antibiotics may shed the bacterium for up to 70 days after recovery and transmit the disease to susceptible animals even though the bacterium may not be detectable by culture (Neef et al., 1994; Songer and Harris, 1978). It is therefore important to develop diagnostic tools sufficiently sensitive to detect pigs that are carriers of the infection.

Culture has a high sensitivity with a detection limit as low as 140 bacterial cells per gram faeces even after

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storage of the faecal swab in Amies media for up to 3 weeks (Fellström et al., 2001). However, CBT are time-consuming and the use of PCR directly on faeces, or applied to primary cultures, may reduce the time needed for *Brachyspira* spp. detection. To date, several PCR systems for *B. hyodysenteriae* and *B. pilosicoli* have been developed (e.g. Atyeo et al., 1999; Elder et al., 1994; Harel and Forget, 1995; Leser et al., 1997; Park et al., 1995) that target defined genes (16S rRNA, 23S rRNA and NADH Oxidase), or undefined genes. In this study, detection and identification of *B. hyodysenteriae* and *B. pilosicoli* were compared by culture and biochemical tests (CBT) and a duplex PCR system targeting the 16S rRNA-gene of *B. pilosicoli* and the *tly*A-gene of *B. hyodysenteriae*.

2. Material and methods

2.1. Samples

Samples consisted of single cotton swabs with faecal matter that were stored in Amies (charcoal) media (Copan, Italy), as well as seeded faeces prepared in Eppendorf tubes (Brand GMBH, Wertheim, Germany). In total, 236 Amies swabs and 76 samples in tubes were analysed for *Brachyspira* spp. Thirty-six of the swabs were prepared under laboratory conditions. Two hundred swabs were submitted by practicing veterinarians to the National Veterinary Institute (SVA) for routine *Brachyspira* laboratory diagnostics. Only samples from diarrhoeic pigs were included in the study, and a maximum of 10 samples from each submission was randomly selected. Samples were sent from throughout Sweden.

2.2. Culture and biochemical tests (CBT)

The swabs were inoculated onto selective *Brachyspira* agar plates (Blood agar base no. 2 [Oxoid], 5% sheep blood, 1% sodium ribonucleate, 800 μg/μl spectinomycin, 25 μg/μl vancomycin and 25 μg/μl colistin; National Veterinary Institute, Uppsala, Sweden) and incubated in an anaerobic atmosphere provided by gas generator envelopes (BBL GasPak Plus; Becton Dickinson, Cockeysville, MD) in jars for 6 days at 42 °C. Subcultures were obtained by inoculating spirochaetal growth with a plastic loop onto fastidious anaerobe agar plates (FAA-plates: Fastidious anaerobe agar [Lab M], 10% horse blood; National Veterinary Institute, Uppsala, Sweden) that were incubated anaerobically for 3 days at 42 °C. Identification of *Brachyspira* sp. was performed on pure *Brachyspira* cultures, based on type of haemolysis and biochemical tests (Fellström et al., 1999).

2.3. PCR

A duplex PCR system was developed based on two primer pairs that detected a 526 bp portion of the *tly*A gene of *B. hyodysenteriae* (Bh tlyA F: 5′-GCA GAT CTA AAG CAC AGG AT-3′, Bh tlyA R: 5′-GCC TTT TGA AAC ATC ACC TC-3′) and a 930-bp portion of the 16S rRNA gene of *B. pilosicoli* (Bp16S F: 5′-CAT AAG TAG AGT AGA GGA AAG TTT TT-3′, Bp16S R: 5′-CTC GAC ATT ACT CGG TAG CAA CAG-3′) (Fellström et al., 1997; Fellström et al., 2001). The compositions of the PCR-mix and amplification temperatures were optimised to obtain optimal conditions for the detection of both of *B. hyodysenteriae* and *B. pilosicoli* (Markoulatos et al., 2002).

The PCR-reaction was carried out in a total volume of 50 μl. For each amplification reaction, 2 μl of the template was added to 48 μl of the reaction mix. The reaction mix consisted of 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 100 μM of each dNTP, 1 U *Taq* DNA polymerase (AmliTaq Gold™) and 0.2 μM of the *tly*A primers and 0.14 μM of the 16S rRNA primers. The mix was complemented with 8% glycerol. Water and glycerol were purchased from Sigma-Aldrich Corporation (Saint Louis, MO) and the remaining ingredients from Applied Biosystem (Foster City, CA). The reaction mixture including template was subjected to 30 cycles of amplification with a hot start of 95 °C for 6 min to activate the AmpliTaq Gold™ enzyme. Each cycle involved denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s and an extension step at 72 °C for 60 s. The amplification was ended with a prolonged extension step at 72 °C for 6 min and the sample was thereafter cooled to 4 °C. The samples were subjected to electrophoresis in 1.5% (wt/vol) agarose gels in 1× TBE buffer at 120 V for 30 min, and then stained with ethidium bromide before being viewed on top of a UV-table and photographed.

Templates were prepared from pure cultures of *B. hyodysenteriae* $B78^T$ (ATCC 27164) or *B. pilosicoli* $P43/6/78^T$ (ATCC 51139), respectively. The bacterial cells were boiled in 50 μl ultra pure water, centrifuged and the supernatant was used as template as previously described by Fellström et al. (2001). One minor modification was made to the protocol; one small loop (1 μl) of bacteria was picked from the agar plate surface and used for each template. Tenfold dilution-series were made from each of the bacterial templates. The dilution series were prepared by adding 5 μl of the template to 45 μl