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Rapid isolation of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* from pigs

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Abstract

The aim of this study was to compare and evaluate the time required to isolate *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* from porcine faeces. This was done using previously described selective media (spectinomycin) S400, (colistin, vancomycin and spectinomycin) CVS and (spectinomycin, vancomycin, colistin, spiramycin and rifampin with swine faecal extract) BJ, compared with the method based on blood agar modified medium, with spectinomycin and rifampin (BAM-SR), including a pre-treatment step. Fourteen spirochaetal strains were obtained in pure cultures after 5 days (48 h in BAM-SR primary plate and three passages every 24 h in brain heart infusion (BHI) without antibiotics) pre-treating simulated samples in brain heart infusion broth with spectinomycin (400 μg/ml) and rifampin (15 μg/ml), before streaking on the selective BAM-SR medium.

Spirochaetes from samples in S400, CVS and BJ, with and without pre-treatment, were obtained in pure cultures only after repeatedly transferring on plates of the same selective medium requiring 15–18 days according to the strain. BAM-SR used after the pre-treatment step showed a detection limit ranging from 3.5×10^2 to 6.7×10^7 cells/g faeces and was the only method able to support the growth of spirochaetes after 48 h.

Keywords: Brachyspira; Rapid isolation; Selective medium; Spirochaetosis; Swine dysentery

1. Introduction

Swine dysentery (SD) is a severe mucohaemorragic diarrhoeal disease that often affects pigs in the final stages of growth, causing persistent diarrhoea and significant financial losses due to mortality, decreased rate of growth, poor feed and costs for antimicrobial therapy. The disease is caused by the strongly beta-haemolytic anaerobic spirochaete *Brachyspira hyodysenteriae*, which has been reported throughout the world (Kunkle and Kinyon, 1988) and first described in Italy in 2001 (Calderaro et al., 2001). Intestinal spirochaetosis (IS), caused by a weakly beta-haemolytic spirochaete belonging to the species *Brachyspira pilosicoli*, is a milder form of colitis and diarrhoea than SD (Taylor et al., 1980). *B. pilosicoli* is considered a

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zoonotic agent and was recognized as causative agent of intestinal spirochaetosis identified in humans and different animal species (Trott et al., 1996).

Several selective media have been described for the isolation of intestinal spirochaetes: (spectinomycin) S400, (colistin, vancomycin and spectinomycin) CVS, (spectinomycin, vancomycin, colistin, spiramycin and rifampin with swine faecal extract) BJ and blood agar modified medium, with spectinomycin and rifampin (BAM-SR) (Songer et al., 1976; Jenkinson and Winger, 1981; Kunkle and Kinyon, 1988; Sanna et al., 1982), but their respective sensitivity levels were not compared. The selective media were based on Trypticase Soy Agar (TSA) supplemented with antibiotics: spectinomycin (Songer et al., 1976); colistin, vancomycin and spectinomycin (Jenkinson and Winger, 1981); and spectinomycin, vancomycin, colistin, spiramycin and rifampin with swine faecal extract (Kunkle and Kinyon, 1988; Achacha and Messier, 1992).

In our laboratory, human intestinal spirochaetes have been isolated using a method developed by us based on BAM-SR medium containing spectinomycin (400 μg/ml) and rifampin (30 μg/ml) (Sanna et al., 1982; Calderaro et al., 1997a), after a pre-treatment step in brain heart infusion (BHI) with spectinomycin and rifampin (Calderaro et al., 2003). This method, based on the pre-treatment of the faecal sample before the streaking in the BAM-SR medium, was of great utility also in isolating intestinal spirochaetes from pigs (Calderaro et al., 2001). Up to now, comparative studies of selective media for the isolation of porcine intestinal spirochaetes (Achacha and Messier, 1992; Songer et al., 1976; Kunkle and Kinyon, 1988) have not investigated sensitivity values nor tested the BAM-SR medium.

The aim of this study was to evaluate the time required to detect porcine spirochaetes using the previously described selective media (S400, CVS and BJ) compared to the BAM-SR based method. The sensitivity (detection limit) of these selective media was investigated too, evaluating the pre-treatment step in BHI-SR to improve the spirochaetal recovering.

2. Strains used in this study

Fourteen porcine strains were used in this study. Seven Swedish isolates of *B. pilosicoli* strains AN914:90, AN497:93, C62 and C162 and *B. murdochii* strains Be236, C301, C378 (Fellström et al., 1997) were kindly provided by Professor Claes Fellstrom. *B. hyodysenteriae* strain 11135/2/98 had been previously isolated in our laboratory (Calderaro et al., 2001). Two reference strains, *B. pilosicoli* strain P43/6/78^T (ATCC 51139) and *B. hyodysenteriae* strain B78^T (ATCC 27164), were used. Four porcine strains (*B. pilosicoli* 3398/00, 6932/2/01, *B. intermedia* 7545/01, *B. murdochii* 13565/3/98) were isolated in this study and subsequently tested with the same protocols in BAM-SR plates only to confirm the results we obtained using Swedish and reference strains.

3. Selective media tested

The following three selective media were used to be tested comparatively to BAM-SR. The medium S400 was prepared with Trypticase Soy Agar (40 g/l), defibrinated bovine blood (5%) and spectinomycin (400 μg/ml) (Songer et al., 1976). The CVS medium was obtained with the same protocol as S400, with colistin (100 U/ml), vancomycin (25 μg/ml) and spectinomycin (400 μg/ml), as reported by Jenkinson and Winger (1981). The BJ medium was produced with Trypticase Soy Agar (40 g/l), freshly thawed pig faecal extract (5%), colistin (6.25 μg/ml), vancomycin (6.25 μg/ml), spectinomycin (200 μg/ml), spiramycin (25 μg/ml), rifampin (12.5 μg/ml) and defibrinated bovine blood (5%), as described by Kunkle and Kinyon (1988). The BAM medium was obtained by using Blood Agar Base no. 2 (Oxoid) (40 g/1), Beef Extract (Difco) (3 g/l) and Bacto Peptone (Difco) (5 g/l), supplemented with defibrinated horse blood (7%) and added with spectinomycin (400 μg/ml) and rifampin (30 μg/ml) (BAM-SR), as previously described (Sanna et al., 1982; Calderaro et al., 1997a, 2001).

4. Seeding of spirochaetes in porcine faecal samples

Fourteen porcine spirochaetes were used to seed into porcine faecal samples not containing spirochaetes. The absence of spirochaetes in the porcine faecal samples used for the seeding of the spirochaetes was assessed by both isolation and PCR procedures