PCR detection of *Brachyspira aalborgi* and *Brachyspira pilosicoli* in human faeces

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**Abstract**

Previously-developed PCR protocols specific for the 16S rRNA gene of the intestinal spirochaetes *Brachyspira aalborgi* and *Brachyspira pilosicoli* were adapted for the detection of these species in human faeces, following DNA extraction and purification using mini-prep columns. The limits of detection in seeded faeces for *B. aalborgi* and *B. pilosicoli* respectively were $2 \times 10^2$ and $7 \times 10^3$ cells per PCR reaction, equivalent to $5 \times 10^4$ and $1 \times 10^5$ cells per g of faeces. The PCR techniques were applied to faecal samples from two patients with histological evidence of intestinal spirochaetosis. In the first patient, in whom *B. aalborgi* had been identified by 16S rDNA PCR from colonic biopsies, a positive amplification for *B. aalborgi* only was obtained from the faeces. The organism could not be isolated from these faeces. In the second patient, both colonic biopsies and faeces were PCR positive for *B. pilosicoli* only, and *B. pilosicoli* was isolated from the faeces. These new faecal PCR protocols should be valuable for future studies on the epidemiology of intestinal spirochaete infections in human populations, particularly as it is not currently possible to isolate *B. aalborgi* from faeces. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Two species of anaerobic intestinal spirochaetes, *Brachyspira* (formerly *Serpulina*) pilosicoli [1] and *Brachyspira aalborgi* [2], colonise the human large intestine [3,4]. Both species are associated with a condition known as intestinal spirochaetosis (IS), in which a thin carpet-like layer of spirochaetes is found attached by one cell end to the colo-rectal surface epithelium [5]. Colonisation of humans by intestinal spirochaetes has been linked to a variety of intestinal disorders, including chronic diarrhoea and rectal bleeding [6].

*B. pilosicoli* has been isolated from human faeces or intestinal biopsies in a number of studies from the USA [7], Europe [8–10], Oman [11], Papua New Guinea [12], and Australia [3,9,13]. It also naturally infects pigs [1], dogs [12], and chickens [14]. In contrast, there are only two published reports of *B. aalborgi* being cultured – from rectal biopsy samples from five human patients in Denmark [2], and a colonic biopsy from a Swedish patient [15]. *B. aalborgi* has never been isolated from faecal specimens. Specific information about the prevalence of *B. aalborgi* came from studies using the polymerase chain reaction (PCR) on DNA extracted from colorectal biopsy samples. It was the most common of the two spirochaete species involved in IS in humans in Norway, the USA and Australia [4,16]. Currently there is a lack of information about the distribution of *B. aalborgi* in human populations, directly related to the absence of reliable means to culture the spirochaete, and a lack of PCR or similar methods to detect the spirochaete in faeces.

The purpose of the current study was to develop PCR procedures for detecting *B. aalborgi* and *B. pilosicoli* in human faeces.

2. Materials and methods

2.1. Source of samples

Approval for this study was obtained from the Sir Charles Gardner Hospital and the Murdoch University...
2.3. Seeding of faeces with B. aalborgi and B. pilosicoli

Tris-HCl, 1 mM EDTA, pH 8.0) to a concentration of 10^9 cells ml^-1. The agar and resuspended thoroughly in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to a concentration of 10^9 cells ml^-1.

2.2. Intestinal spirochaete strains

Two B. aalborgi strains and a single B. pilosicoli strain were obtained from the collection held at the Reference Centre for Intestinal Spirochaetes, Murdoch University, Western Australia. These were B. aalborgi type strain 513^T, a recent Swedish strain W1 provided by Dr C. Fellström, and B. pilosicoli strain WesB, originally isolated from the faeces of an Australian Aboriginal child [13]. The spirochaetes were propagated anaerobically in an atmosphere of 94% N₂ and 6% CO₂ at 37°C on non-selective Trypticase soy agar containing 5% (v/v) defibrinated sheep blood for 15 days. Viable cells were scraped from the agar and resuspended thoroughly in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) to a concentration of 10^9 cells ml^-1.

2.3. Seeding of faeces with B. aalborgi and B. pilosicoli

The sensitivity of the faecal PCRs for B. aalborgi and B. pilosicoli was determined by seeding spirochaetes into faecal samples prior to extraction of chromosomal DNA. Sterile tubes containing either 0.1 or 0.2 g of faeces from three healthy individuals were each resuspended with 200 μl serial 10-fold dilutions of spirochaete cells of either B. aalborgi strain 513^T or W1, or B. pilosicoli strain WesB in TE buffer ranging from 10^3 to 10^8 cells ml^-1. One tube consisting of 200 μl of TE buffer was included as a negative control. These faecal suspensions were then immediately used for column extraction of chromosomal DNA and subsequently for the appropriate PCR.

2.4. Isolation of DNA

Chromosomal DNA was extracted and purified from the seeded faeces and the faeces of the two patients using a JETquick Tissue DNA Spin Column (Genomed GmbH; Bad Oeynhausen, Germany) according to the manufacturer’s instructions, with minor modifications. Briefly, sterile tubes containing 0.1 g of faeces were resuspended with 1 ml TE buffer. The tubes were vortexed for 5 min and centrifuged briefly at 200×g to pellet the large debris. The supernatant was transferred to a new sterile tube and the cells pelleted in a microfuge at 2500 rpm for 10 min before the supernatant was discarded. The pelleted cells were resuspended with 100 μl of TE buffer and lysed by boiling for 5 min. 100 μl of T2 or T2 substitute (4 M GuSCN, 100 mM Tris–HCl pH 7.5) was added to the lysed cells and incubated at 70°C for 10 min. The tube was allowed to cool at room temperature for 1 min before 100 μl of absolute ethanol was added. The entire contents of the tube were applied to a spin column and centrifuged at 6000×g for 1 min. The column was washed once with 500 μl T3 at 6000×g for 1 min and centrifuged at 14000×g for 2 min to remove any residual ethanol. Chromosomal DNA was eluted from the column at 6000×g using 75 μl of TE buffer heated to 70°C. The column eluate was reheated to 70°C and the elution step repeated. The final DNA solution was used for PCR amplification.

2.5. Polymerase chain reactions

A 471 base pair sequence of the 16S rRNA gene of B. aalborgi, equivalent to the base pair positions 172–675 of the 16S rRNA gene of Escherichia coli, was targeted for PCR amplification, as described previously [4], except that dimethyl sulfoxide was not added. Furthermore, the 16S rRNA gene of B. pilosicoli was targeted for PCR amplification as described previously [4], except that a different reverse primer (5’-CCCTACAAATATCCAGACT-3’) was used. This produced a 439 bp product equivalent to the base pair positions 204–676 of the 16S rRNA gene of E. coli [16]. For both PCR reactions, the amplification mixtures consisted of a 23 μl reaction mix of 67 mM Tris–HCl (pH 8.8), 16.6 mM (NH₄)₂SO₄, 0.45% (v/v) Triton X-100, 0.2 mg ml^-1 gelatin, 0.55 U of Tho Plus DNA polymerase (Biotech International Ltd; Bentley, WA, Australia), 1.5 mM of MgCl₂, 5 nmol of each dNTP (Amersham Pharmacia Biotech AB; Uppsala, Sweden) and 12.5 pmol of each primer (F-Ba 16S and R-Ba 16S).

An upper phase of 20 μl of Chill-out 14® liquid wax (MJ Research Inc; Watertown, MA, USA) was added and allowed to solidify before the addition of 2 μl purified DNA solution extracted from faeces. Cycling conditions involved a 2 min denaturing step at 94°C, followed by 33 cycles of denaturation at 94°C for 30 s, annealing at 46°C for 30 s, and a primer extension at 72°C for 30 s. The PCR products were subjected to electrophoresis in 1.5% (w/v) agarose gels in 1×TAE buffer (40 mM Tris–acetate, 1 mM EDTA), stained with ethidium bromide and viewed under UV light.

2.6. Culturing of faecal samples

The seeded faeces, and faecal samples from the two IS patients, were cultured on selective media based upon those described for the isolation of B. aalborgi [2] and B. pilosicoli [17]. Both used Trypticase soy agar (BBL) containing 5% defibrinated ovine blood, with spectinomycin.
Table 1
Patient information and results of PCR detection of intestinal spirochaetes following selective culture, and amplification of DNA extracted from paraffin embedded colonic biopsies and faeces

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex/age in years</th>
<th>Spirochaete isolation from faeces</th>
<th>Species identification by PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M/36</td>
<td>No</td>
<td>N/A&lt;br&gt;B. aalborgi&lt;br&gt;B. pilosicoli</td>
</tr>
<tr>
<td>B</td>
<td>M/30</td>
<td>Yes</td>
<td>B. pilosicoli&lt;br&gt;B. pilosicoli&lt;br&gt;B. pilosicoli</td>
</tr>
</tbody>
</table>

N/A, not appropriate (no spirochaetes isolated).

(400 µg ml⁻¹) and polymyxin (5 µg ml⁻¹) added for the isolation of B. aalborgi and spectinomycin (400 µg ml⁻¹), colistin (25 µg ml⁻¹) and vancomycin (25 µg ml⁻¹) added for the isolation of B. pilosicoli. The plates were incubated in anaerobic jars in an atmosphere of 94% N₂ and 6% CO₂ at 37°C for up to 21 days, and were examined for growth at weekly intervals.

3. Results

3.1. PCR on seeded faeces

The sensitivity obtained for the B. aalborgi specific PCR, as applied to the B. aalborgi strains seeded into faeces from three healthy individuals, ranged from 5×10⁴ to 5×10⁶ per g of faeces. This was equivalent to a range of 2×10² to 2×10³ cells per PCR reaction.

The sensitivity obtained for the B. pilosicoli specific PCR, as applied to the B. pilosicoli strain WesB seeded into faeces from the same three healthy individuals, ranged from 1×10⁵ to 1×10⁷ per g of faeces. This was equivalent to a range of 7×10¹ to 7×10⁵ cells per PCR reaction.

3.2. Patient A

No spirochaetes were isolated from a faecal sample from patient A using the selective culture media, despite apparently viable spirochaete-like organisms being observed by phase contrast microscopy in that sample. However, positive amplification was obtained for the B. aalborgi specific PCR from DNA extracted from the same faecal sample. No amplification was obtained from these DNA extracts when the B. pilosicoli specific PCR was applied (Table 1).

3.3. Patient B

Spirochaetes were cultured from the faecal sample taken from patient B, and were shown to be B. pilosicoli by PCR. DNA isolated from the faecal sample was only amplified in the PCR protocol specific for the 16S rRNA gene of B. pilosicoli. No amplification was obtained from these DNA extracts when the B. aalborgi specific PCR was applied (Table 1).

4. Discussion

The described faecal PCR techniques were shown to detect either B. aalborgi or B. pilosicoli in faeces from patients with diagnosed cases of histological IS. The results correlated with the results of PCR on DNA extracted from colonic biopsies, and with faecal culture (in the case of B. pilosicoli). As anticipated, it was not possible to isolate B. aalborgi from the faeces (Table 1).

The use of spin columns for the extraction of bacterial DNA from faecal samples has been described previously [18]. Compared to the sensitivities obtained in that study, albeit for another species, the detection limits of 2×10² to 2×10⁵ cells per reaction and 7×10¹ to 7×10⁵ cells per reaction obtained in the current study for B. aalborgi and B. pilosicoli respectively were relatively poor, as well as being quite variable. Nevertheless these detection levels are comparable to those obtained by the selective culture of B. pilosicoli from pig faeces, and those obtained where PCR has been applied to primary spirochaetal growth on the isolation plate [19]. Consequently the technique described here should find application for direct detection of B. pilosicoli in faeces samples from humans, and probably also from infected animals. In the case of B. aalborgi, since this species has never been successfully isolated from faeces, the new faecal PCR technique should prove particularly valuable. Although detection limits were not as good as anticipated, modifications such as the use of a nested PCR to the same DNA extraction method may increase the sensitivity of PCR detection.

The PCR techniques described here should prove useful in a diagnostic setting, for example in monitoring faecal shedding of B. aalborgi in patients with histological evidence of IS who are undergoing therapy for the condition. Furthermore the two PCR methods can now be used in prospective epidemiological studies in different populations, allowing the collection of important comparative data about the distribution and disease associations of these two anaerobic spirochaete species.

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References


