Brachyspira Species and Gastroenteritis in Humans

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Brachyspira species have been implicated as a potential cause of gastroenteritis in humans; this is, however, controversial. In 733 gastroenteritis cases and 464 controls, we found 29 samples positive for Brachyspira species (2.3% of cases and 2.6% of controls; \( P = 0.77 \)). Brachyspira species were not associated with gastroenteritis in humans.

**TABLE 1** Primers and probes\(^a\)

<table>
<thead>
<tr>
<th>Primer or probe</th>
<th>Sequence (5′–3′)</th>
</tr>
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<tbody>
<tr>
<td>PhHV Fw</td>
<td>GGGCGAACGACAGTGAATC</td>
</tr>
<tr>
<td>PhHV Rev</td>
<td>CGGTTCAACGTACCA</td>
</tr>
<tr>
<td>PhHV Probe</td>
<td>LC640-TTTTATGGCTGCGCTACATCGATATAGG-BBQ</td>
</tr>
<tr>
<td>Bspf Fw</td>
<td>AACATGGACTAATCCGATATAC</td>
</tr>
<tr>
<td>Bspf Rev</td>
<td>CTCAAGTCGGTTACCTAC</td>
</tr>
<tr>
<td>Bspf Probe</td>
<td>LC640-TGAGCTGTCGGCTATTATTCT-BBQ</td>
</tr>
<tr>
<td>Bpilo Probe</td>
<td>R6G-AAGTAGAAGGAAAGTTTCTCGTCTT-BBQ</td>
</tr>
<tr>
<td>Bhomi Probe</td>
<td>6FAM-TCTTGGACACATAATGCTGAGG-AAAGGGG-BBQ</td>
</tr>
<tr>
<td>Baalb Probe</td>
<td>Cyan500-TGACGCTAATCGGATGAGGAAAGG-BBQ</td>
</tr>
</tbody>
</table>

\(^a\) The PCR protocol consisted of 95°C for 10 min (preincubation) and 45 cycles of 95°C for 10 s (denaturation) followed by 60°C for 60 s (amplification). PCR runs included a negative control (high-performance liquid chromatography-grade water) and positive controls (\( B. aalborgi \) [513A\(^T\]), \( B. pilosicoli \) [P43/678\(^T\]), and a biopsy sample positive for \( B. hominis \)). Primers and probes were synthesized by TIB Molbiol GmbH, Berlin, Germany. PhHV, phocine herpesvirus. Reporter dyes: LC640, LightCycler Red 640; LC601, LightCycler Red 610; R6G, rhodamine 6G; 6FAM, 6-carboxyfluorescein; BBQ, Blackberry quencher.

Human intestinal spirochetosis (HIS) is caused by spirochetes from the genus Brachyspira (1) and is histologically characterized by the apical attachment of these bacteria to the mucosa of the colon (2). Three Brachyspira species have been reported to colonize humans: \( B. aalborgi \), \( B. pilosicoli \), and the provisionally named “\( B. hominis \)” (3–7). Although it is unclear whether HIS is associated with clinical symptoms, there is evidence suggesting an association between \( B. pilosicoli \) and diarrhoea in humans (3, 8–11). However, due to the obligate anaerobic culture requirements for these bacteria, most studies on HIS are based on histopathology without species differentiation, as histopathology requires additional techniques to differentiate between species (3, 12). As a result, the clinical significance of HIS has remained controversial for decades (1, 13–15). Several studies have investigated the prevalence of Brachyspira species in patients with diarrhea, and various incidences have been reported, depending on the investigated population (high- versus low-income countries) and techniques used (culture versus histopathology). In this study, we investigated the prevalence of Brachyspira species in Dutch primary care patients with and those without symptoms of gastroenteritis. For this purpose, we used fecal samples stored from a case-control study reported in 2001 (17). DNA was extracted as previously described (18), directly from the fecal samples without selenite enrichment. Amplification was performed by a previously described real-time PCR amplifying a 142-bp region of the 16S rRNA of all Brachyspira species (3), with some minor modifications. The modifications consisted of three species-specific probes targeting \( B. pilosicoli \), \( B. aalborgi \), and “\( B. hominis \)” and another reverse primer. These probes were validated against a known set of 66 Brachyspira-positive samples (cultured isolates and histopathologically positive samples) and 26 Brachyspira-negative samples (3). The PCR protocol and primer and probe sequences are supplied in Table 1. PCR inhibition was defined as a PhHV cycle threshold value greater than the average of all PCRs plus 2 times the standard deviation. Samples exceeding this value and all Brachyspira-positive samples were reextracted from the original fecal samples. Samples were excluded from analysis if the internal control exceeded the cutoff again. Statistical analyses were performed by using IBM SPSS Statistics version 20 (release 20.0.0) for Macintosh OS X.

Samples from 736 cases and 465 controls were available. Four samples (three cases and one control) had to be excluded due to inhibition of the PCR. Twenty-nine samples were positive for Brachyspira species: 17 cases (2.3%) and 12 controls (2.6%) (\( P = 0.77 \)), determined by a chi-square test. All PCR-positive samples were confirmed after reextraction.

\( B. aalborgi \) was detected in 18 samples (11 cases and 7 controls), and “\( B. hominis \)” was detected in 7 samples (4 cases and 3 controls). Four samples were positive for both \( B. aalborgi \) and “\( B. hominis \)” (two cases and two controls). \( B. pilosicoli \) was not detected in any of the included samples, although this species was responsible for 15.4% of HIS cases in a previous study in the Netherlands using similar methods (3). No statistically significant association (\( P < 0.05 \)) was found regarding the presence of Brachyspira species in cases and controls or regarding age, gender, age, coinfections, urbanization, seasons, year of study, or duration of symptoms.
Other studies investigating the presence of *Brachyspira* species in Western societies used microscopy of colon biopsy specimens taken for various reasons and reported a prevalence between 0.0% and 2.5% (8, 19–22), but those studies did not include a control group or used samples taken for other suspected pathologies as controls. In a culture-based study, 1,018 fecal samples from patients with diarrhea and 509 samples taken for screening purposes were analyzed, and spirochetes resembling *B. pilosicoli* were detected in 8 (0.8%) patients and in 15 (2.9%) of the screening samples (23). However, all positive samples were from either persons of Asian descent (*n* = 16; 12 of whom had recently been to the Indian subcontinent) or homosexuals (*n* = 7), whereas HIS is reported to be more frequent in HIV-infected patients or in men who have sex with men (23–26) and is known to be more prevalent in low-income countries (8, 21, 22, 27, 28). In the present study, one patient was HIV positive (*B. aalborgi* and "*B. hominis*" coinfection), but all but *Brachyspira*-positive patients were native Dutch inhabitants (one was born in Africa but immigrated to the Netherlands 19 years before the original study), and three had been abroad in the week before sampling (eastern Europe, Central/South Africa, and North Africa).

Infections with *Brachyspira* species have been associated with male gender (8, 14, 22), and in the present study there was a non-significant trend toward a higher prevalence of *Brachyspira* species in fecal samples of men (3.5% versus 1.8%; *P* = 0.075, determined by a chi-square test).

The storage of feces in peptone-glycerol at −80°C for more than 10 years is a potential limitation, as this may have had a negative impact on DNA quality. However, PCR-based retesting (18, 29) of 12 fecal samples that were positive in 1997 did not suggest a loss of DNA quality (see Table S1 in the supplemental material). Moreover, if quality had decreased, this would have affected samples from both cohorts equally. One sample was found to be negative for *Dientamoeba fragilis* by PCR, whereas it was microscopically positive in the original study. This is likely due to the fact that *D. fragilis* excretion is known to be highly variable, and routinely, three fecal samples are collected for microscopy on three different days, while only a single fecal sample was stored and available for PCR analysis. Furthermore, seven additional positive results were obtained by PCR, illustrating the higher sensitivity of PCR than conventional diagnostic procedures. In conclusion, these results were indicative of correct preservation of the DNA.

Two important limitations of this study are to be taken into consideration: as our study was performed in general practices, the DNA was microscopically positive in the original study. This is likely due to the fact that *D. fragilis* excretion is known to be highly variable, and routinely, three fecal samples are collected for microscopy on three different days, while only a single fecal sample was stored and available for PCR analysis. Furthermore, seven additional positive results were obtained by PCR, illustrating the higher sensitivity of PCR than conventional diagnostic procedures. In conclusion, these results were indicative of correct preservation of the DNA.

Two important limitations of this study are to be taken into consideration: as our study was performed in general practices in the Netherlands, our findings may not be generalizable to countries with a different prevalence of *Brachyspira* species among humans or to different patient populations, and since no *B. pilosicoli* isolates were identified in this population, this does not allow for conclusions on its pathogenic potential but strongly suggests that it is not relevant as a pathogen in general practice.

This is the first study of the association of *Brachyspira* species and gastroenteritis in humans using prospectively collected fecal samples from patients with and those without symptoms of gastroenteritis. Based on our results, we conclude that *Brachyspira* species are not associated with gastroenteritis in humans in general practice.

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