Comparison of prevalence and risk factors for faecal carriage of the intestinal spirochaetes *Brachyspira aalborgi* and *Brachyspira pilosicoli* in four Australian populations

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SUMMARY

This study examined the prevalence of the intestinal spirochaetes *Brachyspira aalborgi* and *Brachyspira pilosicoli* in different Western Australian (WA) populations. Faecal samples included 287 from rural patients with gastrointestinal symptoms, comprising 142 from non-Aboriginal and 145 from Aboriginal people; 227 from recent healthy migrants to WA from developing countries; and 90 from healthy non-Aboriginal individuals living in Perth, WA. DNA was extracted from faeces, and subjected to PCR assays for both species. *B. pilosicoli*-positive individuals were confined to the rural Aboriginal (14·5%) and migrant (15·0%) groups. *B. aalborgi* was detected at a lower but similar prevalence in all four groups: rural non-Aboriginals, 5·6%; rural Aboriginals, 6·9%; migrants, 7·9%; controls, 5·6%. In migrants and Aborigines, the presence of *B. pilosicoli* and *B. aalborgi* was associated ($P < 0.001$), suggesting that colonization by *B. pilosicoli* may be facilitated by colonization with *B. aalborgi*. Amongst the Aboriginal patients, logistic regression identified both spirochaete species as being associated with chronic diarrhoea, failure to thrive and being underweight. Both species may have pathogenic potential, but *B. aalborgi* appears more host-adapted than the opportunistic *B. pilosicoli*.

INTRODUCTION

Brachyspira aalborgi and *Brachyspira pilosicoli* are related anaerobic intestinal spirochaetes that colonize human beings [1]. Both species can attach by one cell end to the lumenal surface of the colorectal epithelium. Large numbers of attached spirochaetes may form a 'false brush border', and this histologically defined condition is known as 'intestinal spirochaetosis' (IS) [2]. In colorectal biopsies with IS from the general population in developed countries, *B. aalborgi* is more commonly present than *B. pilosicoli* [3, 4]. *B. aalborgi* is difficult to culture due to its fastidious growth requirements and extremely slow growth rate. It is usually detected by polymerase chain reaction (PCR) or gene probe assays, and has only occasionally been isolated [5–7]. *B. pilosicoli* is easier to isolate, and has been cultured from faeces in several developing countries [8–11], from Australian Aborigines [12, 13], migrants to Australia from developing countries [13] and homosexual males [14]. Prevalence rates for *B. pilosicoli* in the faeces of these populations vary from ~10 to 50%. It is rarely identified in other populations [12, 15]. *B. pilosicoli* also colonizes many animal species, and is a well-recognized pathogen of pigs and poultry [16, 17].

Compared to *B. pilosicoli*, there is limited understanding of the distribution of *B. aalborgi*. Most studies on *B. aalborgi* have focussed on its detection in intestinal biopsies. One recent large-scale study

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examined its presence in faecal specimens [11]; PCR was used on DNA extracted from faeces to investigate carriage of both spirochaete species amongst workers on tea plantations in Assam, India. The prevalence of *B. aalborgi* (6%) was considerably less than for *B. pilosicoli* (25%). In a study of Dutch patients with chronic diarrhoea of unknown aetiology, *B. pilosicoli* was not detected using faecal PCR, whilst two of 182 samples (1·1%) were positive for *B. aalborgi* [18]. Currently, the impact on human health caused by colonization with intestinal spirochaetes remains unclear [1].

The purpose of our study was to record and compare the prevalence of *B. pilosicoli* and *B. aalborgi* in different population groups in Western Australia (WA). We used specific PCRs for the two species on DNA extracted from faeces. Most samples previously had been investigated for *B. pilosicoli* by culture [13]. This allowed additional comparison of the usefulness of faecal culture compared to PCR for detection of *B. pilosicoli*, and helped ensure the quality control on the *B. aalborgi* faecal PCR. Potential risk factors for PCR positivity for the two species also were examined using demographic and hospital data relating to health and the presence of potential enteric pathogens.

MATERIALS AND METHODS

Ethics approval

The study was conducted with the approval of the Sir Charles Gardiner Hospital and Murdoch University Human Ethics Committees.

Stool samples examined

In total, 609 stool samples from 604 individuals were used for faecal PCR. Of these, 514 samples from rural patients and migrants had been used in a previous culture-based study of *B. pilosicoli*, and further details of the sources of these samples have been published [13]. The other 36 samples from the previous study (all from rural Aboriginal people) were not used because either they represented multiple samples from the same individual, or there was insufficient sample available. The samples used in the current study comprised 287 from rural WA patients with gastrointestinal symptoms, including 142 samples from non-Aboriginal people and 145 samples from Aboriginal people; 227 consecutive samples obtained by the WA Health Department for routine pathogen screening from healthy recent migrants to WA from developing countries, including multiple samples from members of 49 family groups with two or more individuals tested; and 90 samples from healthy, non-Aboriginal, non-migrant control individuals living in metropolitan Perth. The latter individuals worked at, or were family members of individuals who worked at the University of Western Australia or Murdoch University, and all volunteered to provide samples and personal data. One control individual (C-40) who was PCR positive for *B. aalborgi* provided four additional stool samples at monthly intervals, whilst another positive individual (C-46) provided a second sample after 1 month. All samples were stored at $-80\ ^{\circ}$C until used.

Control strains

B. aalborgi type strain 513A[T] and *B. pilosicoli* type strain P43/6/78[T], obtained from the culture collection at the Reference Centre for Intestinal Spirochaetes at Murdoch University, were used in the PCRs as positive and negative controls. The spirochaetes were propagated anaerobically on non-selective trypticase soy agar containing 5% (v/v) defibrinated sheep blood in an atmosphere of 94% H_2 and 6% CO_2 at 37 °C, for up to 15 days in the case of *B. aalborgi*. Viable cells were scraped from the agar and suspended in TE buffer (10 mM Tris–HCl, 1 mM EDTA; pH 8·0) to a concentration of 10^{11} cells/ml.

PCR assays

The PCRs used for the detection of *B. pilosicoli* and *B. aalborgi* were the same as described previously for identification of the two organisms in human faeces [11, 19], with the primers and PCR conditions being as described by Mikosza and colleagues [3].

DNA extraction from faeces

DNA was extracted from 0·2-g portions of thawed faeces using the QIAmp DNA Stool Mini Kit, according to the manufacturer's instructions (Qiagen GmbH, Hilden, Germany).

Limits of detection by PCR and culture

The limits of detection of the PCRs were investigated using human faeces seeded with the two type strains. Samples were prepared by adding 1 ml of a series of 10-fold dilutions of 1×10^8 to 1×10^3 spirochaetes/ml of TE, in a blinded fashion, to 1 g of faeces from a

healthy donor confirmed free of *B. aalborgi* and *B. pilosicoli* by culture and PCR. The suspensions were thoroughly vortexed to mix. The seeded samples were used fresh, and/or stored at $-80\,^{\circ}$C until being extracted. The effect of storage on DNA in seeded samples was tested by comparing the limits of detection of freshly prepared samples with samples stored at $-80\,^{\circ}$C for over 3 months. The limits of detection of the PCRs also were assessed on their own, using freshly prepared whole cell suspensions at 10^8–10^1 spirochaetes/ml in TE, of which $2.5\,\mu l$ was added directly to reactions. All procedures were performed in duplicate.

The lower limit of detection of *B. pilosicoli* in seeded faeces by culture was assessed by inoculating selective medium comprising trypticase soy agar supplemented with 5% defibrinated ovine blood and $400\,\mu g$/ml spectinomycin, and incubating the plates at $42\,^{\circ}$C for 10 days in an anaerobic jar (94% H_2 and 6% CO_2) [13]. Spirochaetes recovered from the plates were identified on the basis of their morphology, and their reaction in the 16S rDNA PCR.

Sequencing of PCR products

The sequences of 24 *B. pilosicoli* and 22 *B. aalborgi* 16S rRNA gene PCR products were generated with their respective forward and reverse primers. Samples for sequencing were selected from all the positive population groups. Amplified products were purified using a MoBio Ultra Clean PCR clean up DNA purification kit (MoBio Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. DNA in the eluent was sequenced using an ABI PRISM dye terminator cycle sequencing ready reaction kit (ABI Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. The sequence data were aligned and compared with the sequences of the 16S rRNA genes of *B. pilosicoli* strains P43/6/78[T] (GenBank accession no. U23032) and WesB (U23034), and *B. aalborgi* strains 513A[T] (Z22781) and W1 (AF200693), using Biomanager with the ANGIS server (Sydney, NSW, Australia). The *B. aalborgi* sequences were compared to differentiating nucleotide positions identified in previous phylogenetic analyses of *B. aalborgi* 16S rDNA sequences [20, 21].

Demographic and other patient data

Demographic data, and data on the presence of other enteric pathogens previously have been published for the non-control patients [13]. The control individuals from Perth were administered a short questionnaire. Questions included the individual's age, gender, occupation, how long they had lived in Australia, recent travel history, number of individuals in the family, health status, recent antibiotic treatments, and whether they owned pets.

Analysis

The prevalence and 95% confidence interval (CI) were calculated for each species of intestinal spirochaete overall and for each population group. Associations between the presence of the spirochaete species and host and environmental factors were initially made using a Yates corrected χ^2 or a Fisher's exact test with one-tailed P value, depending on the expected population size. Logistic regression analysis then was performed, again separately for each population group and *Brachyspira* spp. Reasons for clinical investigation in the Aboriginal and non-Aboriginal rural patient groups were modelled independently of risk factors and the presence of other organisms. Only variables significant at $P \leqslant 0.25$ in the univariate analysis were considered eligible for inclusion in the logistic regression models. Backward elimination was used to determine which factors could be dropped from the model. The likelihood-ratio χ^2 statistic was calculated to determine the significance at each step of the model building. The level of significance for a factor to remain in a final model was set at 10%. Statistical comparisons were performed using Statistix for Windows (Analytical Software, Tallahassee, FL, USA), Excel 97 (Microsoft Corporation, Redmond, WA, USA), and Egret for Windows (version 2.0.3, Cytel Software, Cambridge, MA, USA).

RESULTS

Limits of detection in seeded faeces

The respective 16S rRNA gene PCRs could detect 1×10^6 cells of *B. pilosicoli* strain P43/6/78[T] and 1×10^5 cells of *B. aalborgi* strain 513A[T] cells in 200-mg faecal samples. This corresponded to DNA from 4.2×10^2 and 4.2×10^1 organisms per reaction tube respectively. Storage of duplicate seeded faecal preparations at $-80\,^{\circ}$C for over 3 months followed by DNA extraction did not reduce the level of detection in either reaction. The lowest concentration of whole cells in TE which allowed PCR amplification was

2·5–25 cells per reaction tube for *B. pilosicoli* and 25 cells per reaction for *B. aalborgi*.

Selective culture of *B. pilosicoli* was capable of detecting fewer bacteria than PCR, with isolation possible from seeded faeces containing 1×10^2 to 2×10^3 colony-forming units (c.f.u.)/g.

Comparison of culture and PCR for detection of *B. pilosicoli* in different populations

Samples from 514 of the patients that were analysed for *B. pilosicoli* by faecal PCR previously had been analysed by culture [13]. All samples from the previously identified culture-positive individuals (14 Aboriginal and 24 migrant) were positive by PCR for *B. pilosicoli*, and an additional 17 samples that had been culture negative were *B. pilosicoli* PCR positive. The new samples comprised seven from Aboriginal Australians, and 10 from migrants. Using PCR, the overall prevalence for *B. pilosicoli* became 14·5% (21/145) amongst Aboriginal patients, 15% (34/227) amongst migrants, and remained zero for rural non-Aboriginal patients and the urban control group. The sensitivity of PCR for the detection of *B. pilosicoli* compared to culture of the organism was 100%, with a specificity of 96·8%; the sensitivity of culture compared to PCR was 69·1%, and the specificity was 100%.

Risk factors for *B. pilosicoli* detection

The detection of additional *B. pilosicoli*-positive individuals by PCR altered the significance of only a few risk factors in univariate analysis. In the previous culture study, *B. pilosicoli* carriage was significantly increased in Aboriginal children aged 2–5 years, and in migrants from the Middle East and Africa, with carriage being significantly associated with detection of faecal protozoa in both groups [13]. Using PCR, in the Aboriginal group *B. pilosicoli* carriage was no longer associated with age group, detection of *Giardia intestinalis*, *Hymenolepsis nana* or protozoa generally. In the migrant group, carriage was no longer significantly associated with country of origin or detection of any parasite or protozoan. In both population groups the only new risk factor for *B. pilosicoli* was the detection of *B. aalborgi*, which was more common in individuals in whom *B. pilosicoli* was detected ($P < 0.001$).

In the logistic regression model of risk factors and organisms associated with *B. pilosicoli* detection in Aborigines using faecal PCR, the odds ratio (OR) for *B. pilosicoli* detection was 11·8 if *B. aalborgi* also was detected (95% CI 2·6–53·3, $P = 0.001$), and was 10 if *Blastocystis hominis* also was detected (95% CI 1·4–69·6, $P = 0.02$). Amongst migrants, the OR for *B. pilosicoli* detection was 21 if *B. aalborgi* was detected (95% CI 6·5–68·6, $P = 0.001$), 6·5 when *Iodamoeba bütschlii* was detected (95% CI 1·4–29·7, $P = 0.015$), and 3·4 in individuals who were not of European origin (95% CI 1·2–9·4, $P = 0.016$).

The logistic regression model created for reasons for investigation in the Aboriginal population identified the odds of *B. pilosicoli* colonization being 10·9 times greater if 'possible worms?' had been given as a reason for investigation (95% CI 1·6–76·5, $P = 0.016$), and 5·5 times greater if any of chronic diarrhoea, failure to thrive (FTT), or being underweight were the reasons for the sample submission (95% CI 1·7–18·1, $P = 0.005$). A stable model could not be generated for *B. pilosicoli* in migrants.

Detection of *B. aalborgi* in control individuals

Five of the 90 (5·6%) control individuals in metropolitan Perth were positive for *B. aalborgi* by PCR. Positive individuals had not taken antibiotics in the previous month. There was no significant difference in risk factors for the detection of *B. aalborgi* in samples from positive and negative individuals. No *P* value was $\leqslant 0.25$, therefore the data were not modelled by logistic regression. The two individuals who had more than one sample collected at monthly intervals were both positive at all sampling times.

Detection of *B. aalborgi* in rural non-Aboriginal Australians

Eight of the 142 (5·6%) rural non-Aboriginal patients were positive for *B. aalborgi*. All positive patients were aged $\geqslant 18$ years, and four were $\geqslant 60$ years. Five colonized patients were from the South West region of WA. All eight positive patients were suffering from diarrhoea, and this was more frequent than in *B. aalborgi*-negative patients (90/134, $P = 0.048$). No stable logistic regression models could be produced for this population.

Detection of *B. aalborgi* in Aboriginal Australians

B. aalborgi was detected in the faeces of 10 out of 145 (6·9%) Aboriginal patients, and both spirochaetes

were detected in six (4·1%) individuals. Only one *B. aalborgi*-positive individual was <2 years of age, whilst two of the individuals colonized by both species were >60 years of age. Colonization with *B. aalborgi* or both spirochaetes together was not affected by patient gender, geographical location, rainfall distribution, or stool consistency.

Four Aboriginal patients in whom *B. aalborgi* was detected were hospitalized, and included two of the patients in whom *B. pilosicoli* was detected – a child <2 years with gastrointestinal symptoms, and an adult with chronic renal failure, eosinophilia and chest pain. The other two patients were a 5-year-old child with eosinophilia and possible worms, in whom *H. nana* was detected, and an adult with suspected *Giardia* infection.

In logistic regression, the OR for *B. aalborgi* detection was 6·6 if the patient was >2 years (95% CI 0·8–52·3), and 5·4 if a parasite was detected (95% CI 1·3–22·7, $P = 0.021$). A number of reasons for investigation remained within the model, including eosinophilia (OR 10·2, 95% CI 0·7–154), suspected *Giardia* infection (OR 8·8, 95% CI 0·7–110·5), chronic diarrhoea, FTT or being underweight (OR 4·9, 95% CI 0·9–28·6), and eosinophilia, weight loss, being underweight and possible worms (OR 19·1, 95% CI 3·0–123·1, $P = 0.002$). A stable model could not be generated for the detection of both organisms in this population group.

Detection of *B. aalborgi* in migrants to WA

B. aalborgi was detected in 18 out of 227 (7·9%) migrant samples, and both spirochaetes were detected in 12 (5·3%) samples. Carriage of *B. aalborgi* or both *Brachyspira* spp. was not associated with country of origin. No specific age group had significantly more individuals with *B. aalborgi* or both organisms detected. The only organisms that were associated with carriage of *B. aalborgi* were *B. pilosicoli* ($P < 0.001$) and any protozoan ($P = 0.014$). No logistic regression model could be generated for the detection of *B. aalborgi* or both organisms.

Twelve of the *B. aalborgi*-positive individuals belonging to seven family groups. Single positive individuals were found in five families, two positive individuals came from a family of four, and five positive individuals were from a family of six. Both *Brachyspira* spp. were detected in samples from individuals from the latter two families; in two individuals from the family of four, and in four of the

Table 1. *Departures of four* B. pilosicoli *16S rDNA sequences from that of the type strain P43/6/78^T in the region equivalent to base positions 243–652 in* E. coli

Sequence	Change	Base position
A-47	C→T	248
M-61	C→T	307
M-62	C→T	307
A-160	Insertion of a C	Between 326 and 327

A, Aboriginal Australian; M, migrant; C, cytosine; T, thymine.

five individuals from the family of six. In addition, both species were detected in two of the five families containing single positive individuals.

Sequencing of PCR products

Sequences of 24 *B. pilosicoli* PCR products from migrants (M) and Aboriginal Australians (A) were analysed. A region of 254 bp was sequenced for all PCR products, and a region of 386 bp was generated for most products. The main sequence pattern was 100% homologous to the sequence of *B. pilosicoli* P43/6/78^T in the region equivalent to 243–652 bp of *E. coli* 16S rDNA. Minor departures occurred in four sequences (Table 1).

Sequences from 12 *B. aalborgi* PCR products from migrants, Aboriginals and non-Aboriginal (NA) individuals, and all 10 PCR products from the five positive control group individuals (C) were analysed. A region of 344 bp was sequenced for all products, with a region of 423 bp assessed for most PCR products. The sequence of most PCR products was 100% homologous to that of *B. aalborgi* 513A^T in the region equivalent to 210–629 bp of *E. coli* 16S rDNA. Small departures existed in sequences of five strains, as shown in Table 2. PCR product sequences from volunteer C-40 in five successive months were all identical in this region, as were the two samples from control individual C-46.

The *B. aalborgi* type strain and the strains from this study with identical sequences corresponded to *B. aalborgi* 'cluster 1', as identified in previous phylogenetic analyses [20, 21]. The difference in the sequence of NA-96 at bases 416, 436, 585 and 587 suggested it was a 'cluster 3' strain, while strains C-07 and M-197 also demonstrated changes in common with this cluster. The cytosine replacement at base 214 in NA-96 and C-03 was consistent with them being either 'cluster 2' or 'cluster 3' strains.

Table 2. *Departures of five* B. aalborgi *16S rDNA sequences from that of the type strain 513^T in the region equivalent to base positions 210–629 in* E. coli

Sequence	Change	Base position
NA-96	T→G	214
NA-96	G→T	416
NA-96	T→C	436
NA-96	T→G	585
NA-96	T→G	587
C-07	G→T	416
C-07	T→C	436
C-03	T→C	214
M-197	G→T	416
NA-131	Insertion of a T	Between 530 and 531

NA, Non-Aboriginal; C, control group; M, migrant; G, guanine; C, cytosine; T, thymine.

DISCUSSION

Using the faecal PCR methodology, levels of detection of both *Brachyspira* spp. from seeded faeces corresponded to 1×10^5 to 1×10^6 organism/g faeces, whilst isolation of *B. pilosicoli* was possible from faeces containing 1×10^2 to 2×10^3 c.f.u./g. Despite this difference in detection level on seeded faeces, faecal PCR for *B. pilosicoli* appeared to be at least as effective as culture in detecting naturally infected individuals. Not only did the PCR detect all the culture-positive individuals, it also detected additional *B. pilosicoli*-positive people. A possible explanation could be that the *B. pilosicoli* cells in the samples that were PCR positive but culture negative were non-viable. The PCR methodology for *B. aalborgi* detected slightly fewer seeded spirochaetes than the *B. pilosicoli* PCR, although the limits of detection for the two species were broadly comparable. Hence, the faecal PCR methodology appeared effective for determining the comparative prevalence of the two species.

Sequence analysis of the PCR products confirmed the specificity of the reactions, and most sequences from a given species were identical. For *B. pilosicoli*, four PCR products differed at one nucleotide from the type strain sequence, consistent with the known heterogeneity in the species [1]. For *B. aalborgi*, all except five strains had an identical sequence to the cultured strains 513A^T and W1, which corresponded to cluster 1 described by Pettersson et al. [20]. The other five strains, representing samples from 29% (5/17) of the individuals from whom product was sequenced, may have been from cluster 2 or 3.

Currently, the comparative epidemiology and pathogenic potential of members of these different clusters is unclear.

The use of PCR for detection of *B. pilosicoli* added relatively little to the conclusions derived from a previous investigation in WA based on culture of these samples [13]. Again, colonization was restricted to the Aboriginal and migrant groups, but with the apparent prevalence of faecal shedding increased to levels more consistent with that previously described in Aboriginal children in a remote rural community in WA (33%) [12], and to those of villagers in Papua New Guinea (23%) [9], India (25%) [11], and Bali, Indonesia (12%) [10]. Apart from the presence of *B. aalborgi*, which was significantly associated with the presence of *B. pilosicoli* in both population groups, and in the previous study from India [11], the new analysis did not strengthen the identification of significant risk factors. It was unfortunate that data were not available on water supply or water quality, as in previous studies in both India and Bali the use of untreated well water was associated with increased *B. pilosicoli* carriage [10, 11]. Differences in water quality might have helped explain the different prevalence observed amongst population groups in this study.

Results for the Aboriginal patient group provided some indirect evidence that *B. pilosicoli* can have a pathogenic role. Four of the newly identified patients were in hospital at the time of sampling, and two were infants with gastrointestinal symptoms but without other pathogens being detected. Logistic regression identified diagnoses of 'possible worms', and chronic diarrhoea, FFT and being underweight as being significantly associated with the presence of *B. pilosicoli*. Chronic diarrhoea is a symptom that has been associated with IS in a number of human studies [22–24].

A major new finding in this study was the identification of *B. aalborgi* in almost equal proportions in the faeces of the four population groups examined (5·6–7·9%). This rate was similar to the 6% found in villagers in India [11]. Furthermore, carriage in migrants was not affected by their country of origin. These data suggest that *B. aalborgi* is distributed at a similar prevalence in many human populations throughout the world. Its distribution was quite different from that of *B. pilosicoli*, which was found only in Aboriginal and migrant individuals, and which occurred at a higher prevalence (∼15%).

Attempts to identify specific risk factors that might explain the different distributions of the two related

spirochaete species met with limited success. Generally there was a lack of consistency between the populations in the risk factors that were associated with *B. aalborgi* detection, despite the populations having a similar prevalence. In part, this inconsistency may have been due to the small number of positive individuals overall, as well as to the lack of uniformity in the criteria used for sample selection from the different groups.

The pathogenic significance of *B. aalborgi* could not be resolved in this investigation. First, *B. aalborgi* was detected in similar proportions in samples from ill (rural) and healthy individuals. Detection was not associated with the presence of gastrointestinal symptoms in control individuals, nor with loose or wet stool consistency in any population group. In addition, detection of *B. aalborgi* was significantly associated with different reasons for investigation in the two ill rural population groups. Detection of *B. aalborgi* was associated with diarrhoea in rural non-Aboriginals, whilst amongst Aboriginals it was significantly associated with a number of other reasons for investigation, including chronic diarrhoea, eosinophilia, weight loss, being underweight and suspected worms. It was not know whether *B. aalborgi* was a cause of these symptoms, or was excreted as a result of symptoms arising for some other reason.

Consistent with earlier suggestions [25], the similar prevalence of *B. aalborgi* in all population groups suggests that it could be a commensal. This possibility is supported by the finding that control individual C-40 was colonized by a strain of *B. aalborgi* over a period of at least 5 months, whilst apparently remaining healthy. On the other hand, *B. aalborgi* was found in rural patients with gastrointestinal symptoms in whom no other pathogenic microorganisms were detected. A similar situation has been reported in other investigations [3, 26, 27]. It is possible that different strains or 'groups' [20] of *B. aalborgi* may vary in their virulence, or that the extent or site of colonization may influence the clinical outcome. To investigate these possibilities, it would be useful to develop animal models in which to test the virulence of different *B. aalborgi* isolates.

Putting the available data together, we hypothesize that the slow growing and fastidious *B. aalborgi* is a human-adapted species, with generally low pathogenic potential. It may be present in low or undetectable numbers in many individuals throughout the world, although it can overgrow and cause clinical signs in some people under some circumstances.

As with other members of the intestinal commensal microbiota [28], *B. aalborgi* may induce an immune tolerance that allows it to persist attached to or in close proximity to the colorectal mucosa for extended periods. The spirochaete is probably transmitted by the faecal–oral route, but also there is potential for spread by ingestion of water or food that is contaminated by human faeces. In contrast, *B. pilosicoli* is not specifically human adapted, and has greater pathogenic potential in humans (and animals). Humans are infected by indirect routes such as exposure to water contaminated by the faeces of animals, birds or infected humans, as well as by faecal–oral cycling amongst human groups living in close proximity in conditions of poor hygiene. In this respect the transmission resembles that of many intestinal protozoa. In those population groups where exposure to *B. pilosicoli* occurs, prior colonization by *B. aalborgi* is a risk factor for colonization, perhaps because of a pre-existing tolerance that occurs to the related spirochaete. This situation tends to limit the generation of protective immune responses against *B. pilosicoli*, which in turn enhances its ability to colonize. Clearly, these hypotheses require further experimental investigation to determine their validity.

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DECLARATION OF INTEREST

None.

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