Isolation of the anaerobic intestinal spirochaete *Brachyspira pilosicoli* from long-term residents and Indonesian visitors to Perth, Western Australia

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Brachyspira pilosicoli is an anaerobic spirochaete that colonizes the large intestine of humans and various species of animals and birds. The spirochaete is an important enteric pathogen of pigs and poultry, but its pathogenic potential in humans is less clear. In the current study, the occurrence of *B. pilosicoli* in faecal samples from 766 individuals in two different population groups in Perth, Western Australia, was investigated by selective anaerobic culture. Of 586 individuals who were long-term residents of Perth, including children, elderly patients in care and in hospital and individuals with gastrointestinal disease, only one was culture positive. This person had a history of diverticulitis. In comparison, faeces from 17 of 180 (9.4 %) Indonesians who were short- or medium-term visitors to Perth were positive for *B. pilosicoli*. The culture-positive individuals had been in the city for between 10 days and 4.5 years (median 5 months). Resampling of subsets of the Indonesians indicated that all negative people remained negative and that some positive individuals remained positive after 5 months. Two individuals had pairs of isolates recovered after 4 and 5 months that had the same PFGE types, whilst another individual had isolates with two different PFGE types that were identified 2 months apart. Individuals who were culture-positive were likely to have been either colonized in Indonesia before arriving in Perth or infected in Perth following contact with other culture-positive Indonesians with whom they socialized. Colonization with *B. pilosicoli* was not significantly associated with clinical signs at the time the individuals were tested, although faeces with wet-clay consistency were 1.5 times more likely (confidence interval 0.55–4.6) than normal faeces to contain *B. pilosicoli*.

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INTRODUCTION

The anaerobic intestinal spirochaete *Brachyspira pilosicoli* colonizes the large intestine of a range of species, including humans, pigs, chickens, dogs and horses (Hampson *et al.*, 2006). *B. pilosicoli* is considered to be a common and important enteric pathogen in pigs and chickens worldwide (Hampson & Duhamel, 2006; Hampson & Swayne, 2008), but its role as a potential human pathogen is less well documented. The spirochaete colonizes and attaches by one cell end to the human colo-rectal epithelium to form a false brush-border (Lee & Hampson, 1994; Trivett-Moore *et al.*, 1998; Mikosza & Hampson, 2001), and has been isolated from the bloodstream of debilitated patients (Fournié-Amazouz *et al.*, 1995; Trott *et al.*, 1997b; Kanavaki *et al.*, 2002). Uncertainty about the clinical signs associated with colonization in humans remains, since most studies on intestinal spirochaetosis in humans have not differentiated between colonization with *B. pilosicoli* or the related *Brachyspira aalborgi*. In some studies where *B.*

pilosicoli has been identified and the health status of the individual has been recorded, colonization has been associated with a number of non-specific problems, including chronic diarrhoea, and failure to thrive and being underweight in children (Lee & Hampson, 1992; Brooke *et al.*, 2006).

Colonization with *B. pilosicoli* is common (10–50 % prevalence) in people living in developing countries, including Gulf Arabs in Oman (Barrett, 1990), villagers in Papua New Guinea (PNG) (Trott *et al.*, 1997a), villagers and peri-urban residents in Bali, Indonesia (Margawani *et al.*, 2004) and villagers in tea plantations in India (Munshi *et al.*, 2004). In developed countries, including the UK and Australia, the spirochaete is rarely isolated from individuals in the general population (Tompkins *et al.*, 1986; Brooke *et al.*, 2006), although prevalence rates reach the high levels seen in developing countries in Australian Aborigines living in remote communities (Lee & Hampson, 1992; Brooke *et al.*, 2001, 2006), homosexual males and HIV patients (Käsbohrer *et al.*, 1990; Trivett-Moore *et al.*, 1998) and immigrants on arrival from developing countries (Brooke *et al.*, 2001, 2006).

Abbreviations: CI, confidence interval; PNG, Papua New Guinea.

To date, there have been no specific surveys to determine whether carriage of *B. pilosicoli* is increased in other groups of potentially more susceptible people. The current study therefore aimed to determine the prevalence of *B. pilosicoli* in such groups in Perth, Western Australia, including children attending day-care centres, elderly patients in hospitals and nursing homes and individuals with gastrointestinal complaints. Furthermore, in view of the high prevalence of colonization with *B. pilosicoli* previously recorded in Indonesians living in Bali (Margawani *et al.*, 2004), a second aim was to investigate whether there was an increased rate of *B. pilosicoli* carriage by Indonesians who were short- or medium-term visitors to Perth.

METHODS

Approvals. The collection of faecal samples was approved by the Murdoch University Human Ethics Committee. The purpose of the study was explained to the participants or their parents or carers, and they signed a consent form.

Sampling. Participants or their carers were provided with 70 ml sterile sample jars with a scoop attached to the lid (Sarstedt). They were asked to collect a faecal sample before 9 am. Samples were collected from the nappies of children at day-care centres and from nappies or bedpans of elderly patients in institutions. The samples were stored on ice for transport to the laboratory on the same day. At the time of sampling, information was provided on the participant's age, gender, health status and any medication taken in the past month. The consistency of the faeces was also subjectively recorded by the investigators as being dry to slightly moist ('normal'), bulky, moist and sticky ('wet clay') or loose and watery ('watery').

Faecal culture. Faeces were plated onto a selective medium (Jenkinson & Wingar, 1981) consisting of trypticase soy agar (Becton Dickinson) with 5% (v/v) defibrinated ovine blood, 400 µg spectinomycin ml⁻¹ and 25 µg ml⁻¹ of each of colistin and vancomycin (Sigma-Aldrich). The plates were incubated for 5–7 days at 37 °C in an environment of 94% H₂ and 6% CO₂, generated with anaerobic GasPak plus sachets (Becton Dickinson). The plates were examined for the presence of a low, flat, spreading growth and associated haemolysis. Surface growth was picked off, resuspended in PBS and examined under a phase-contrast microscope. The surface growths on plates were subjected to a species-specific PCR for *B. pilosicoli*, as previously described by Mikosza *et al.* (1999). Isolates were resuspended in Kunkle's anaerobic broth (Kunkle *et al.*, 1986) and stored frozen at –80 °C.

Source of samples. A total of 1024 faecal samples were collected from 766 individuals. Of these, 586 were long-term residents of Perth in four categories. Category 1 comprised 102 children attending eight child-care centres, with ages ranging from 6 months to 4 years. Fifty-seven children were resampled 1 month later and a further 18 were resampled after another month. Category 2 was elderly and/or disabled people. These included 55 (age range 62–96 years) who were hospitalized in a Department of Rehabilitation and Aged Care facility, 19 of whom were resampled after 3 months, and 70 (age range 45–98 years) from four nursing homes. Eighteen of the latter individuals were resampled three times at intervals of 3–6 weeks. Category 3 comprised individuals with gastrointestinal complaints of suspected microbial origin whose samples had been submitted to a diagnostic laboratory in Perth by their medical practitioners. Information on the patients' ethnicity, age or gender was not available. Category 4 was made up of 72 individuals from the general community, including

university lecturers, members of their families (including five children), students and 14 Indonesian people from four families who were permanent residents of Australia. Ages ranged from 7 months to 71 years. A faecal sample from a dog belonging to a positive individual also was cultured.

The other major group, consisting of 180 people, were Indonesians who were short- to medium-term term visitors to Perth (age range 2–64 years). These individuals were divided into three categories. Category 1 consisted of 99 trainees attending Perth for a short visit of between 1 and 3 months. They were all university or college graduates who visited Perth in groups of 20–30 people. Category 2 included four Indonesians who independently visited Perth for a short period of between 14 and 30 days. Category 3 included 77 postgraduate students and their family members. Sixteen were living alone and the rest were living in 18 families of two or more people. The students were from various universities and cities in Indonesia, but individuals frequently visited each other's homes and socialized. Thirty-six individuals who were initially negative for *B. pilosicoli* were resampled 1 month after the first collection. Eight were resampled a third time, and three were resampled for a fourth, fifth and sixth time. Fourteen individuals who were initially culture-positive were also resampled at monthly intervals. Four were sampled twice, 10 three times, two four times and one five times.

PFGE. All the available *B. pilosicoli* isolates that were recovered were subjected to typing using PFGE using the method described previously (Margawani *et al.*, 2004).

Statistical analysis. Statistix (ver 7.0, Analytical Software) was used to analyse the data. One-way analysis of variance was used to test continuous data between positive and negative individuals. For data within categories, the χ^2 test for independence or the Fisher's exact test were used. Odds ratios and their 95% confidence intervals (CIs) were calculated to determine the association of risk factors and positivity. The prevalence and 95% CIs were also calculated.

RESULTS AND DISCUSSION

B. pilosicoli amongst long-term residents of Perth

Amongst the samples from 586 long-term residents, only one was positive by culture and PCR. This came from one of the 72 (1.4%) faecal samples collected from the general population (category 4). The positive individual was a 60-year-old female of Caucasian background who had lived most of her life in Perth. At the time of sampling she was reported to have influenza-like symptoms and she had suffered from diverticulitis. It was unclear whether these complaints were linked to the colonization, although an association between diverticulitis and intestinal spirochaetosis has been reported previously (Lima *et al.*, 2005). The woman had a pet dog in her house but a faecal sample taken from the animal was negative for *B. pilosicoli*. She had no recent history of overseas travel, although her husband frequently visited and worked in rural areas in Indonesia. At the time of sampling, her husband was culture-negative, but it is possible that he had been infected and transmitted the spirochaete to his wife.

The failure to detect *B. pilosicoli* in the other groups of long-term residents was consistent with previous studies that have demonstrated that *B. pilosicoli* is uncommon in

the general population in Western countries, even in individuals with evidence of gastrointestinal disease (Goossens *et al.*, 1983; Tompkins *et al.*, 1986; Lee & Hampson, 1992; Brooke *et al.*, 2001, 2006). The individuals sampled in the current study deliberately included children and the elderly, but the study showed that even these potentially more susceptible groups are rarely colonized.

***B. pilosicoli* prevalence in Indonesian visitors to Perth**

In contrast with the long-term residents, *B. pilosicoli* was cultured from 17 of the 180 samples (9.4%) from Indonesian visitors. Positive individuals included six of the 99 short-term trainees (6.1%) and 11 of the 77 postgraduate students and family members who were medium-term visitors (14.3%) (Table 1). Previously, Indonesians in Bali were shown to have overall *B. pilosicoli* prevalence rates of around 12%, with the prevalence at different locations varying from 3 to 23% (Margawani *et al.*, 2004). Hence, the prevalence amongst the visitors resembled those found in Indonesia.

Indonesians who were positive for *B. pilosicoli* had resided in Perth for between 10 days and 4.5 years (median 5 months) and the negative individuals from 1 week to 4.5 years (median 10 weeks). There was no significant difference between the length of stay and colonization ($f=2.5$; $df=1$, 179; $P=0.11$). The mean age of the Indonesians who were positive for *B. pilosicoli* (27.5 years)

was significantly lower than the people who were negative (35.7 years) ($f=5.43$; $df=1$, 179; $P=0.021$), and people who were 18 years or younger were 3.7 times (95% CI 1.2–11) more likely to be positive (22.2%) than those over 18 years of age (7.1%) ($\chi^2=6.06$; $df=1$, 1; $P=0.014$). This finding is consistent with the results of a study in Aborigines in the northern part of Western Australia (Lee & Hampson, 1992) and in people from Oman (Barrett, 1990), where it was shown that colonization was significantly more common between the ages of 2 and 18 years than in other age groups. Young people may have both greater exposure to and increased opportunity for transmission of *B. pilosicoli* due to their close physical contact with each other.

Five of 62 (8.1%) females and 12 of 126 (10.2%) males were positive for *B. pilosicoli*. Although males were 1.3 times more likely to be colonized than females (95% CI 0.4–3.9), this difference was not significant ($\chi^2=0.21$; $df=1$, 1; $P=0.65$). Similar findings have been observed in villagers from PNG (Trott *et al.*, 1997a), Australian Aborigines (Lee & Hampson, 1992) and migrants to Australia from Asia, the Middle East and Africa (Brooke *et al.*, 2001, 2006).

Amongst the postgraduate group, 10 positive individuals belonged to seven (39%) of the 18 families, whilst the other positive individual was one of the 16 who lived alone (6%). This difference in prevalence was not significant ($\chi^2=0.4$; $df=1$, 1; $P=0.53$). Of the 36 negative individuals from the postgraduate group who were resampled, all were

Table 1. Demographic information relating to the 17 Indonesians in Perth who were culture-positive for *B. pilosicoli*, including, where available, the PFGE type of the isolate recovered

Other family members providing samples were negative. NA, Not appropriate, these individuals visited Perth alone. NT, Not typed.

Individual*	Age (years)	Gender	Family	No. in family	Time in Perth†	PFGE type‡
1	25	Female	NA	NA	1 month	7
2	35	Male	NA	NA	1 month	6
3	35	Male	NA	NA	2 months	1
4	45	Male	NA	NA	3 months	NT
5	40	Male	NA	NA	1 week	NT
6	38	Male	NA	NA	1 week	8
7	38	Male	A	4	5 months	4, 4
8	36	Female	A	4	5 months	NT
9	5	Female	A	4	5 months	NT
10	43	Male	B	6	3 years	3, 3
11	10	Female	B	6	4.5 years	NT
12	15	Male	C	4	18 months	NT
13	10	Male	D	6	5 months	NT
14	13	Male	E	4	16 months	4, 5
15	38	Female	F	2	5 months	NT
16	4	Female	G	3	2 years	NT
17	39	Male	NA	NA	5 months	2

*Individuals 1–6 were short-term trainees.

†Length of time since last visit to Indonesia.

‡Where more than one PFGE type is shown, this refers to different isolates obtained from the same individual at different sampling times.

still negative 1 month after the initial sampling, and eight were still negative 2 months later. Only three of the eight individuals provided further samples, and these were still negative at 3, 4 and 5 months after the initial sampling. These results were consistent with there being no significant external source of *B. pilosicoli* infection in Perth. On the other hand, 11 of 14 people (78.6%) who were positive at the initial sampling were still positive 1 month later. Two months later, of the eight people tested, five remained positive and three had become negative. At the third sampling, two of the three positive sampled individuals who were available for testing remained positive and the other had become negative. One individual was available for further sampling and was still positive at the fifth sampling. These results suggest that some of the positive individuals were colonized for an extended period.

Two households had more than one family member positive for *B. pilosicoli*. The first consisted of a mother, a father and two sons (6 and 10 years old). All provided faecal samples and three were positive (the mother, the father and the 6-year-old son). The father remained positive for at least 5 months and the son and the mother for at least 1 month. The elder son was negative on all four samplings. The second family consisted of a father, a mother and five children. Only the father and two children provided faecal samples. The father and his 10-year-old daughter were positive for up to 2 months, but they did not provide further samples.

To gain further insight into transmission routes, isolates were subjected to PFGE. Unfortunately only 11 isolates were successfully recovered from -80°C storage, and these were separated into eight PFGE types (Table 1). Two isolates recovered 5 months apart from a 38-year-old Indonesian man in family A were identical (PFGE type 4), as were two samples taken 4 months apart from a 43-year-old man in family B (PFGE type 3). The latter individual had not been outside Perth for 3 years, and it is possible that he had been infected in Perth, perhaps from other Indonesians. Evidence for such potential transmission could be seen from the fact that an isolate from a 13-year-old boy in family E had the same PFGE pattern (type 4) as that of the man in family A, especially as it was known that members of these two families frequently socialized. Furthermore, another isolate taken from the boy 2 months later had a different PFGE type (type 5).

In the month prior to the survey, diarrhoea (16.7%) and abdominal pain (14.3%) were the most common symptoms reported by the respondents. However, the presence or absence of spirochaetes was not significantly related to the presence of any of the clinical symptoms reported. Two of the 17 individuals who were positive for *B. pilosicoli* reported mild influenza-like illness and headache in the week immediately preceding the survey, and these symptoms resemble those previously reported in a volunteer who had drunk a culture of *B. pilosicoli*

(Oxberry *et al.*, 1998). Six respondents reported taking antibiotics in the week immediately preceding the sampling; none of these were positive. Most faeces (140; 77.8%) appeared normal and the remainder (40; 22.2%) had a wet-clay consistency. Twelve (8.6%) of the normal and six (12.5%) of the wet-clay faeces were positive. Although there was no significant difference between prevalence in faeces of these consistencies ($\chi^2=0.6$; $df=1$; $P=0.45$), wet-clay faeces were 1.5 times more likely than normal faeces (95% CI 0.5–4.6) to contain *B. pilosicoli*. An association between wet faeces and colonization by *B. pilosicoli* has been seen previously in Australian Aborigines (Lee & Hampson, 1992; Brooke *et al.*, 2006) and in one of two sampling sessions in villages from both PNG (Trott *et al.*, 1997a) and Bali (Margawani *et al.*, 2004).

In conclusion, *B. pilosicoli* is uncommon amongst the general long-term population of Perth, including the young and elderly, but it is relatively common amongst short- to medium-term visitors from Indonesia. In countries such as Australia, *B. pilosicoli* should be considered in the differential diagnosis when treating people with diarrhoea or mild influenza-like symptoms who are short-term visitors or recent migrants from developing countries.

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