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SHORT PAPER

Carriage of intestinal spirochaetes by humans: epidemiological data from Western Australia

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SUMMARY

The purpose of this study was to investigate carriage of intestinal spirochaetes by selected population groups in Western Australia. Stool specimens from 293 rural patients with gastrointestinal disorders, and from 227 healthy migrants from developing countries were cultured. Spirochaete isolates were identified using PCR, and typed by pulsed field gel electrophoresis (PFGE). *Brachyspira aalborgi* was not isolated. *Brachyspira pilosicoli* was recovered from 15 rural patients, all Aboriginal. Prevalence was 9·9% in 151 Aboriginals and 0% in 142 non-Aboriginals. Carriage of *B. pilosicoli* amongst migrants was 10·6% (24/227). Carriage was significantly increased in Aboriginal children aged 2–5 years ($P = 0{\cdot}0027$) and in migrant individuals from the Middle East and Africa ($P = 0{\cdot}0034$). Carriage was significantly associated with detection of faecal protozoa in both Aboriginals ($P = 0{\cdot}0021$) and migrants ($P = 0{\cdot}012$). PFGE results indicated that the *B. pilosicoli* strains were genetically diverse.

Two species of intestinal spirochaete colonize humans: *Brachyspira aalborgi* and *Brachyspira* (*Serpulina*) *pilosicoli* [l, 2]. Both have been isolated from biopsies showing histological evidence of 'intestinal spirochaetosis' (IS), where large numbers of spirochaetes are attached end-on to the lumenal surface of the large intestinal epithelium, forming a characteristic false brush border [3]. Both species are slow-growing anaerobes requiring specialized isolation media. *B. aalborgi* has only been isolated in two studies, both involving colonic biopsies taken from Scandinavian patients [1, 4]. Recent studies using the polymerase chain reactions (PCR) on DNA extracted from colorectal biopsy specimens with histological evidence of IS found that *B. aalborgi* was the species involved in nearly all cases [5, 6]. These patients were from Western countries, including Australia, and had gastrointestinal symptoms. Consequently, *B. aalborgi* appears to be a common cause of IS in Western countries.

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Carriage of *B. pilosicoli* appears to be relatively uncommon in humans in Western countries and in urban settings [6–8], except in HIV positive patients and homosexual males where rates of 30–50% have been recorded [2, 9]. On the other hand, in developing countries such as Papua New Guinea [10] and Oman [11] *B. pilosicoli* has been isolated from human faeces at a prevalence of approximately 30%. In Western Australia (WA), rural Aboriginal children but not urban non-Aboriginal children are frequently colonized by *B. pilosicoli* [7]. It is not known whether this difference is due to rural living, or other factors. Similarly, although individuals from developing countries are frequently colonized by *B. pilosicoli*, it is not known whether migrants to WA from such countries continue to carry the organisms. This study therefore was undertaken to determine the prevalence

Table 1. *Origin and microbiology results for individuals colonized with* B. pilosicoli, *and their PFGE patterns*

PFGE Type	Isolate	Gender/age at sampling (y)	Microbiological result	Nationality/location
1	WHP22†	F/5	*Chilomastix mesnili* *Blastocystis hominis*	Afghani
2	WHP23†	F/6	*B. hominis*	Afghani
3	WHP18	M/37	Negative	Serbian
4	WHP29	M/7	*B. hominis* *Giardia intestinalis* *Iodamoeba bütschilii*	Ethiopian
5	WHP33	F/20	*B. hominis* *Entamoeba coli*	Ethiopian
6	Gap401	M/adult	NR	Sydney, control strain
7	WHP14	F/12	*G. intestinalis* *Hymenolepsis nana*	Aboriginal, Kimberley
8	WHP03	F/1	*G. intestinalis*	Aboriginal, Kimberley
9	WHP34	M/56	Negative	Serbian/Croatian
10	WHP12#*	F/2	*B. hominis* *C. mesnili* *Trichomonas hominis*	Aboriginal, Kimberley
11◆	WHP16	M/77	*Ent. coli* *Endolimax nana*	Aboriginal, Kimberley
11	167	Child	NR	Aboriginal, control strain
12	WHP26	M/36	*B. hominis*	Serbian
13	WHP24‡	F/21	Negative	Somali
14	WHP21†	F/41	*I. bütschlii*	Afghani
15	WHP28	M/9	*B. hominis*	Ethiopian
16	WHP36	M/15	Negative	Eritrean
17◇	WHP30§	M/5	Negative	Kurd
17	WHP31§	M/43	*B. hominis* *Ent. coli*	Kurd
18	WHP27	F/20	*B. hominis* *Entamoeba hartmanni*	Afghani
19	WHP10	F/68	Negative	Aboriginal, Kimberley
20	WHP39	F/16	*G. intestinalis* *Ent. coli* *I. bütschlii*	Burmese
21	WHP04	F/3	*G. intestinalis* *H. nana*	Aboriginal, Gascoyne
22	WHP37	M/10	*B. hominis* *Ent. coli* *Ent. hartmanni* *I. bütschlii*	Iranian
23	WHP38	M/38	Negative	Afghani
24	WHP25‡	M/1	Negative	Somali
25	WHP01	F/3	*B. hominis* *G. intestinalis* *H. nana*	Aboriginal, Pilbara
26	WHP40	M/33	Negative	Burmese
27	WHP17	M/23	*Ent. coli*	Iraqi
28	WHP05	M/83	*H. nana*	Aboriginal, Kimberley
29	WHP19	M/3	Negative	Ethiopian
30	WHP32	M/22	*B. hominis*	Somali
31◇	WHP02	F/20	Negative	Aboriginal, Kimberley
31	WHP07	F/9mo	*G. intestinalis* *Salmonella hvittingfoss*	Aboriginal, Kimberley

Table 1. (*cont.*)

PFGE Type	Isolate	Gender/age at sampling (y)	Microbiological result	Nationality/location
31	WHP08	M/2	*B. hominis* *Salm. tennessee* *Strongaloides stercoralis* *Trichuris trichuria*	Aboriginal, Kimberley
31	WHP09	M/4	*B. hominis*	Aboriginal, South Eastern
31	WHP11*	M/2	Negative	Aboriginal, Kimberley
31	WHP13	F/1	*T. hominis*	Aboriginal, Kimberley
32	WesB	Child	NR	Aboriginal, control strain
33	WHP20	F/12	Negative	Eritrean
34	31B	Child	NR	Omani, control strain
35◆	WHP06#*	F/2	*Aeromonas caviae*	Aboriginal, Kimberley
35	H21	Child	NR	Aboriginal, control strain
36	WHP35	F/19	Negative	Eritrean
37	P43/6/78†	Pig	NR	Porcine type strain

NR, Not recorded.
†, ‡, §, these isolates were obtained from migrant individuals within the same families.
these isolates were obtained from the same individual.
* these isolates came from Aboriginal patients from the same community.
◆ isolates within these PFGE types were identical when typed by *Sma*I.
◇ isolates within these PFGE types were not identical when typed by *Sma*I.

of carriage of intestinal spirochaetes in rural individuals and in migrants to WA. It was conducted with the approval of the Sir Charles Gardiner Hospital and the Murdoch University Human Ethics Committees.

Stool samples ($n = 550$) were selected from those sent to the Enteric Laboratory at the Western Australian Centre for Pathology and Medical Research (PathCentre) for routine testing between September 1998 and July 1999. Samples included 323 specimens from 293 rural patients who were being investigated for gastrointestinal complaints and 227 samples from 227 migrants from developing countries who were screened for faecal pathogens on entering Australia.

All specimens underwent routine examination for bacterial pathogens and intestinal parasites in the Enteric Laboratory at PathCentre, according to current protocols. For isolation of spirochaetes, faeces were streaked onto two selective Trypticase Soy Agar plates supplemented with ovine blood (5%) and containing spectinomycin (400 μg/ml) (S medium) or spectinomycin (400 μg/ml) and polymyxin B (5 μg/ml) (SP medium). S medium was incubated at 42 °C in an anaerobic jar (94% H_2 and 6% CO_2) for 14 days and SP medium at 36 °C in an anaerobic chamber (80% N_2, 10% H_2, 10% CO_2) (Don Whitley Scientific Ltd, Shipley, Yorkshire) for 28 days. Plates were examined for spirochaete growth every 3–5 days until 14 days, and then at weekly intervals. Spirochaetes identified by colonial and phase-contrast microscopic morphologies were subcultured onto blood agar (BA) (Columbia agar) base with 5% horse blood (5–10 days anaerobic incubation), and stored at −70 °C in 50% horse serum/1% brain heart infusion broth/10% glycerol.

Spirochaete identity was confirmed using *B. pilosicoli* and *B. aalborgi* specific PCR designed to amplify portions of the 16S rRNA gene of each organism [6]. The *B. aalborgi* PCR was cycled at an annealing temperature of 48 °C, rather than 46 °C.

Isolates of *B. pilosicoli* and six control strains were analysed by PFGE (Table 1), using minor modifications to a method developed for *Staphylococcus aureus* [12]. Digestion was with 50 units of *Mlu*I (Biotech International Ltd.) or *Sma*I (Promega). Electrophoresis was at 200 V for 20 h with pulse time ramped at 1–40 s for *Mlu*I digested plugs, followed by 2 h at 45–60 s for *Sma*I digested plugs. Images were scanned using a Biorad Gel Doc 2000+ and analysed using Biorad Molecular Analyst Software. The programme created a dendrogram from a matrix of Pearson correlation coefficients by the unweighted pair group method of arithmetic averages (UPGMA) clustering fusion strategy.

Details of patient demographics were obtained from request forms accompanying samples. Statistical

analysis was carried out using χ^2 or Fisher's exact (FE) tests to compare proportions within groups.

Forty isolates confirmed as *B. pilosicoli* were obtained. Isolate, patient and sample characteristics are summarized in Table 1. The isolates came from 24 of the 227 (10·6%) migrants and from 16 samples from 15 (5·1%) of the rural patients, all of whom were Aboriginal. The rural patients consisted of 151 Aboriginals and 142 non-Aboriginals. Prevalence amongst the Aboriginal patients therefore was 9·9%, while overall prevalence of colonization was 7·5%. It appears from these data that living in rural areas in WA is not itself a risk factor for infection with *B. pilosicoli*. Rather, colonization appears linked to factors associated with Aboriginality. In turn, this may reflect the generally low socio-economic status and poor living conditions of rural Aborigines.

Patients originated from various regions throughout WA, however, 80% (12/15) of colonized individuals came from the Kimberley region. This is consistent with a previous study in WA where intestinal spirochaetes, later identified as *B. pilosicoli*, were found in the faeces of 32·6% of predominantly children in a rural community of Aboriginals in the Kimberley [7].

No significant gender related difference in *B. pilosicoli* carriage was found and isolations were made at the same relative incidence in all months tested. The age distribution was different for the migrant and Aboriginal groups, but this reflected different population age distributions. The age distribution for migrants approximated a normal distribution and the ages of positive migrant individuals was not significantly different from this distribution. Conversely, nearly half (47%) of the Aboriginal samples came from children under 2 years of age, reflecting the high incidence of infectious diarrhoea in Aboriginal children from birth to 3 years [13]. Nevertheless, significantly more isolates ($\chi^2 = 11{\cdot}00$; $P = 0{\cdot}0027$) came from patients between 2 and 5 years of age than from < 2 year old or > 5 year old individuals. This again agrees with the earlier findings of Lee and Hampson [7]. Three children in this 2–5 year age group were in-patients of childrens' wards in regional hospitals when samples were obtained. In addition, a child from whom two isolates were obtained was hospitalized at the time the second sample was taken.

Amongst migrants, *B. pilosicoli* isolates were obtained from 25% (2/8) of Asian, 3·2% (3/94) of Eastern European, 13·8% (9/65) of Middle Eastern and 20·0% (10/50) of African individuals. Significantly more cases came from the Middle East and Africa than from Eastern Europe ($\chi^2 = 8{\cdot}622$; $P = 0{\cdot}0034$). Unfortunately, it was not known whether some migrants had spent time in transit camps, where they may have been infected, rather than in their countries of origin.

Some migrants were grouped by family, and 48 family groups were tested. Average family size was 3·3, with a range of 2–6. Seven families had 1 positive individual, while 3 contained multiple positive individuals. Two isolates were obtained from a family group of 3 members, 2 from a family group of 5 members, and 3 isolates came from a family of 6.

It was difficult to assess whether *B. pilosicoli* had a pathological role in the individuals in this study, due to the frequent presence of multiple enteric pathogens and the lack of a healthy control group. In both cohorts, the isolation of *B. pilosicoli* was associated with the presence of parasites (Aboriginal (FE; $P = 0{\cdot}0014$); migrant ($\chi^2 = 4{\cdot}37$; $P = 0{\cdot}0037$)), or protozoa (Aboriginal (FE; $P = 0{\cdot}0021$); migrant ($\chi^2 = 6{\cdot}24$; $P = 0{\cdot}012$)), regardless of their pathogenic status. Within the Aboriginal group, carriage of *B. pilosicoli* was significantly associated with detection of another organism listed in Table 1 ($\chi^2 = 8{\cdot}59$; $P = 0{\cdot}003$). Specifically, detection of *G. intestinalis* (FE; $P = 0{\cdot}024$), *Hymenolepis nana* (FE; $P = 0{\cdot}012$) or *Blastocystis hominis* (FE; $P = 0{\cdot}006$) was associated with carriage of *B. pilosicoli*. In the seven (46·7%) positive children from whom other known pathogens were isolated, the significance of *B. pilosicoli* was unclear. In patients where no other pathogen was found, a stronger argument can be made for a possible role of *B. pilosicoli* as a cause of disease. These patients included the 4 hospitalized children and 4 who were over 20 years of age, 3 of whom were elderly. Although only a few samples were obtained from elderly patients, the high prevalence in this group may reflect a reduced immune response leading to an increased susceptibility to the organism.

In migrants, *B. pilosicoli* was obtained from significantly more samples containing an organism of uncertain pathogenic potential than from entirely negative samples ($\chi^2 = 4{\cdot}871$; $P = 0{\cdot}027$). *B. pilosicoli* carriage was significantly associated with *B. hominis* ($\chi^2 = 4{\cdot}73$; $P = 0{\cdot}030$), *Entamoeba coli* (FE; $P = 0{\cdot}036$) and *Iodamoeba bütschlii* (FE; $P = 0{\cdot}005$).

Previously, both *Giardia* [14] and *Balantidium coli* [15] have been suggested to have synergistic relationships with intestinal spirochaetes. In a study of homosexual men [16], spirochaete carriage was

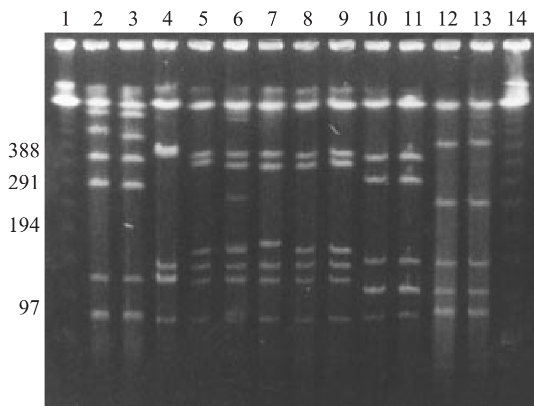

Fig. 1. PFGE banding patterns obtained from *Sma*I digestion of isolates in types 17, 31, 11, 35. Lambda DNA size standards (kb) are in lanes 1 and 14. Isolates WHP30, WHP31, WHP02, WHP07, WHP08, WHP09, WHP11, WHP13, 167, WHP16, H21 and WHP06 are shown in lanes 2–13. WHP30 and WHP31 differ by one band at approximately 440 kb. WHP07, WHP11 and WHP13 (lanes 5, 8 and 9) are identical while WHP02, WHP08 and WHP09 differ from this pattern. The pairs of isolates 167 and WHP16 (lanes 10 and 11) and H21 and WHP06 (lanes 12 and 13) are each identical.

associated with detection of 3–5 unspecified 'non-pathogenic protozoa'. It is uncertain how intestinal spirochaetes and protozoa may interact. Infection with one organism could have caused flushing out of the other, one or other organism may have initiated a predisposition to infection, or a dual role in colonization or disease could be occurring. Further investigation into the interactions between protozoa and *B. pilosicoli* infections is required.

PFGE was carried out on only 39 of the 40 *B. pilosicoli* isolates, as isolate WHP15 could not be recovered from storage. Digestion of DNA with *Mlu*I gave 9–16 DNA bands. Using this enzyme, 37 distinct banding patterns were obtained for the 39 isolates plus 6 control isolates, confirming previous observations that *B. pilosicoli* is genetically heterogeneous [17–19]. Isolates from Aboriginals, migrants and control strains were widely distributed throughout the dendrogram produced from analysis of *Mlu*I patterns (not shown).

PFGE types 11, 17, 31 and 35 each contained multiple isolates and were analysed further using *Sma*I (Fig. 1). All other patterns represented a single strain. Isolates in types 11 and 35 could not be differentiated by *Sma*I digestion. These types contained control strains H21 and 167, previously isolated from Aboriginals in 1989 from near Fitzroy Crossing in the Kimberley region. These were each identical to strains WHP06 and WHP16 respectively, isolated in the current study from Aboriginal patients from near Derby in the Kimberley region. Generally there were no other obvious geographical or temporal relationships observed among the distribution of isolates in the dendrogram.

The two isolates of Type 17 differed by a single band with *Sma*I. These originated from two migrant individuals from the same family. All other strains from migrant family groups were unrelated. It appears that transmission within families may occur, but is not necessarily common. Type 31 contained six isolates collected at different times of year from six Aboriginals living in a variety of geographical areas. After analysis by *Sma*I, WHP02, WHP08 and WHP09 demonstrated minor departures from the main pattern exhibited by the other three isolates. Previous PFGE studies of human and animal strains have found multiple isolates within the same type to be linked geographically [17–19], but this is the first report of a widely disseminated strain or cluster of strains approximating a clonal group.

Isolates WHP06 (type 35) and WHP12 (type 10) from the same Aboriginal child were unrelated. Previous studies have determined that it is possible for individuals to be colonized by more than one strain of *B. pilosicoli* [18]. The child was either re-infected with another strain, or was colonized by more than one strain. The remaining isolate from that community (WHP11) was unrelated to either WHP06 or WHP12, but was one of the six isolates in Type 31.

Although *B. aalborgi* was not isolated in this study, since it has fastidious growth requirements it could have been present but undetected. It has never previously been isolated from faeces, only from colorectal biopsies. Spirochaetes were observed by wet mount microscopy in 69·2% (27/39) of samples from which an isolate was obtained. However, another 10 samples (3 from Aboriginals and 7 from migrants) contain spirochaetes that were not recovered by culture (sensitivity 69·2%, specificity 97·0%), and some of these may have been *B. aalborgi*. Recently, PCR methods have been developed to detect *B. aalborgi* in the faeces [20], and in the future it would be useful to apply this technique to investigate the prevalence of *B. aalborgi* in the different population groups.

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