Intestinal spirochaetes colonizing Aborigines from communities in the remote north of Western Australia

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SUMMARY

Intestinal spirochaetal bacteria were isolated from 59 of 181 (32.6%) faecal samples obtained from Aboriginal children and a few adults living in communities in the Kimberley region in the north of Western Australia. Colonization was more common in young Aborigines between 2 and 18 years of age than it was in adults, or in babies and children less than 2 years of age. Three of 22 Aboriginal children who were sampled on two consecutive years were colonized on both occasions. None of four other children were found to be consistently colonized with the bacteria when sampled on three sequential years, but three were positive on two consecutive visits and the other child was positive on the first and third sampling. Most Aboriginal children had abnormal or watery stools, and both abnormal and watery stool samples were significantly more likely to contain spirochaetes than were normal samples. However, it was not possible to prove that the spirochaetes were the cause of the diarrhoea. In contrast, spirochaetes were only recovered from 8 of 695 (1.2%) faecal samples that were obtained from other mainly non-Aboriginal children and adults in Western Australia or the Northern Territory of Australia, even though most of these individuals were suffering from gastrointestinal disturbances.

INTRODUCTION

A variety of spirochaetal bacteria colonize the intestinal tracts of humans [1]. In this paper the term ‘intestinal spirochaete’ is reserved to describe a group of anaerobic spirochaetal bacteria that are 4–20 μm long, 0.2–0.5 μm in diameter and are irregularly coiled. These spirochaetes grow within 1–2 weeks as a thin film on trypticase soy agar supplemented with 5% ovine or bovine blood, 400 μg/ml spectinomycin, and 25 μg/ml each of colistin and vancomycin. Bacteria of this sort that have been recovered from the pig have been placed in the new genus Serpulina [2, 3], containing the species Serpulina (Treponema) hyodysenteriae and Serpulina (Treponema) innocens. Serpulina hyodysenteriae is strongly beta-haemolytic and is the aetiological agent of a major endemic disease of pigs called swine dysentery [4]. Infection results in colonic malabsorption and dehydration, with diarrhoea or a mucoid dysentery. Weakly beta-haemolytic porcine isolates

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have been called *S. innocens* [5]; whilst most of these are considered to be non-pathogenic, certain isolates have been associated with a condition known as ‘spirochaetal diarrhoea’, which resembles mild swine dysentery [6-8]. Spirochaetes that are similar to *S. innocens* of the pig have frequently been observed in human faeces, and have been cultured in several studies [9-12]. A possible role for these bacteria in production of gastrointestinal disturbances and diarrhoea has been suggested in some studies [9, 10, 13, 14], but the pathological significance of intestinal spirochaetes in general has been questioned by other workers [12, 15, 16]. Colonization is apparently more common in Africans, Arabs and Indians than it is in Western populations living in the United Kingdom [11, 12, 17, 18].

The purpose of the present study was to determine whether Australian Aboriginal children living in remote communities had an increased risk of colonization with intestinal spirochaetes as compared to the general population of Australia.

**MATERIALS AND METHODS**

*Source of specimens*

Faecal samples were obtained from seven different sources. Firstly a total of 181 samples were obtained from Aboriginal children and 17 adults (Table 1) living in nine communities within a 100 kilometre radius of the settlement of Fitzroy Crossing in the Kimberley Region in the north of Western Australia. These samples were obtained on three visits, in mid-1989, 1990 and 1991. The communities contained approximately 630 individuals. Samples were only obtained with the approval of the individuals or their parents, using the assistance of local public health workers. Only 17 samples were obtained from individuals older than 18 years because generally adults were unwilling to supply stool specimens. Four of the children were sampled on all 3 visits and another 22 were sampled on 2 consecutive visits.

At the time of collection these stool samples were subjectively divided into three categories. Well-formed samples were recorded as ‘normal’. Samples that were unformed, moist and/or had a greasy appearance were scored as being ‘abnormal’, and samples that were loose and watery were recorded as being ‘watery’.

The second source of faecal samples (*n* = 127) was from non-Aboriginal children under 10 years of age in the suburbs of Darwin, in the Northern Territory of Australia. The children had various gastrointestinal disturbances, and their stool samples had been submitted to a pathology service for microbiological examination by local medical practitioners. The third and fourth sources of faecal samples (*n* = 186 and 36 respectively) came from two other private diagnostic laboratory services. These samples were obtained from individuals of various ages (2 months to 78 years of age), most of whom were living in the Perth metropolitan area of Western Australia. These samples were all from individuals with a history of various gastrointestinal disorders.

The fifth source of stool samples (*n* = 268) came via the Western Australian State Public Health Laboratories. These samples were obtained from individuals of various ages (6 months to 62 years of age), and were submitted by medical
practitioners or local hospitals for microbiological and parasitological examination. All the samples that were examined for spirochaetes had initially been shown to be positive for *Giardia duodenalis*. Approximately two-thirds of the samples came from individuals living in the Perth metropolitan area, the rest came from individuals in either the southwest or in the Kimberley (northern) regions of Western Australia.

The sixth source of stool samples (*n* = 39) was from children under 10 years of age who were in hospital in Perth. All had gastrointestinal disorders that had necessitated admission to hospital.

The seventh source of specimens (*n* = 39) was from healthy children aged between 7 months and 11 years, all of whom were living in the Perth metropolitan area and whose parents either worked at or had associations with people who worked at the School of Veterinary Studies, Murdoch University.

*Culturing for spirochaetes*

Cotton-tipped swabs were inserted into the centre of each stool sample and were used to inoculate trypticase soy agar (BBL) plates containing 5% defibrinated bovine blood, 400 μg/ml spectinomycin and 25 μg/ml each of vancomycin and colistin. After streaking out, the plates were incubated at 37 °C in anaerobic jars in an atmosphere of 94% H₂ and 6% CO₂ for 5–21 days before being examined.

All the stool samples from the Aboriginal communities were plated out on the same day that they were collected: a subset of 49 samples was also transported to Perth by car, and held in closed specimen containers at 4 °C for 15 days before being plated out again. The faecal samples obtained from sources two to five were 1–15 days old (mean 8.3 days) when they were plated out. These samples were held at 4 °C prior to them being sent to our laboratory for examination. The samples from the sixth and seventh sources were plated out on the day of collection.

*Characterization of isolates*

Spirochaetes were subcultured onto purity plates, and the extent of beta-haemolysis after 5 days incubation was recorded. Spirochaetes were also subcultured into tubes containing prereduced anaerobic sterilized medium [19], and production of indole was assessed for 63 of them after 2–3 days incubation on a shaking platform at 37 °C. Two ml of broth containing actively growing spirochaetes was first extracted by shaking with 1 ml of xylol, and then 4 drops of Kovacs reagent was added. A red ring formed in the top of the broth in positive cultures. The biochemical activity of 14 of the Australian isolates and of human

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**Table 1. Age distribution of Australian Aborigines examined for intestinal spirochaetes**

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>&lt;2</th>
<th>2–3</th>
<th>4–5</th>
<th>6–9</th>
<th>10–18</th>
<th>&gt;18</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number examined</td>
<td>47</td>
<td>59</td>
<td>28</td>
<td>21</td>
<td>9</td>
<td>17</td>
<td>181</td>
</tr>
<tr>
<td>Number positive</td>
<td>6</td>
<td>28</td>
<td>11</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>59</td>
</tr>
<tr>
<td>Percent positive</td>
<td>12.8</td>
<td>47.5</td>
<td>39.3</td>
<td>33.3</td>
<td>44.4</td>
<td>17.6</td>
<td>32.6</td>
</tr>
</tbody>
</table>
intestinal spirochaetal strains HRM1, HRM2 and HRM7, that were originally isolated in from human patients in Italy [9], were also examined using the API ZYM System (API, Montalieu-Vercieu, France) [20].

**Statistical analysis**

The statistical significance of differences between groups was assessed using the Chi squared test.

**RESULTS**

**Characterization of spirochaetes**

Spirochaetes grew as a diffuse flat film surrounded by weak beta-haemolysis. Their presence was confirmed by examining surface scrapings from the plate under phase contrast microscopy. Their size and morphology were consistent with both those reported for *Serpulina (Treponema) innocens* of the pig [5], and human intestinal spirochaetes described in the UK [11], and from Gulf Arab populations [12]. Thirty of the 63 samples that were tested (47·6%) produced indole. The biochemical profiles of 17 isolates that were assayed in the API-ZYM system are recorded in Table 2. The profiles of seven Australian isolates (profiles 14.0.4.10.0 and 14.0.12.10.0) were similar to those of *S. hyodysenteriae*, whilst the other 10 isolates, including the 3 from Italy more closely resembled *S. innocens* [20]. Six of the 7 isolates with profiles similar to *S. hyodysenteriae* produced indole.

**Recovery of spirochaetes**

Spirochaetes were cultured from 59 of the 181 Aboriginal samples (32·6%), and from 8 of the 268 samples obtained through the W.A. Public Health Laboratories (3·0%). Records were incomplete for these 8 individuals, but the average age of 6 of them was 23 months (range 16–29 months). Five came from the north of the state, and two from Kalgoorlie in the centre of the southern region. Two children from the north were known to be Aborigines, one was a Caucasian. No spirochaetes were isolated from the other 427 stool samples, so that overall only 8 of 695 samples (1·2%) from sources other than the particular remote Aboriginal communities were found to contain intestinal spirochaetes. There was no statistical difference between the number of males and females colonized ($\chi^2 = 0·97$).

A comparison of culturing Aboriginal stool samples on the day of sampling or 15 days later showed that, of 49 samples that were positive on the first day, 47 (95·9%) still contained viable spirochaetes 15 days later. The age distribution of Aboriginals that were colonized is demonstrated in Table 1. Colonization was significantly more common in Aborigines between 2 and 18 years than it was in those less than 2 years of age ($\chi^2 = 13·39; P < 0·001$). Although based on relatively few samples, there was also a significant tendency for adults to be less commonly colonized than young Aborigines between 2 and 18 years of age ($\chi^2 = 3·91; P < 0·005$). Twenty-two children were sampled on two consecutive annual visits; 12 were not colonized on either visit, 7 were colonized on only one of the two visits, and 3 were positive on both visits. None of 4 other children sampled on 3 consecutive visits was positive on all 3 occasions. However, faecal specimens from 3 children contained spirochaetes on 2 consecutive visits, and the fourth child was positive on the first and third visit.
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Table 2. API ZYM profile of 14 intestinal spirochaetes isolated from humans in Australia and 3 strains from Italy

<table>
<thead>
<tr>
<th>Enzyme activities assayed in API-ZYM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of isolates</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>7</td>
</tr>
</tbody>
</table>


Table 3. Description of 181 stool samples obtained from the Aboriginal communities, and recovery of spirochaetes from the specimens

<table>
<thead>
<tr>
<th>Classification of stool samples*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Spirochaetes present</td>
</tr>
<tr>
<td>Spirochaetes absent</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

* For description see text.

Stool consistency

The consistency of stool samples obtained from the Aboriginal communities and the presence or absence of spirochaetes in the samples are recorded in Table 3. Ninety-eight of the 181 (54.1%) samples were considered ‘abnormal’ at the time of sampling, and 45 (24.9%) were recorded as being ‘watery’. Only 3 of 38 (7.9%) ‘normal’ samples contained spirochaetes, whilst 31 of the 98 (31.6%) ‘abnormal’ samples and 25 of the 45 (55.6%) ‘watery’ stool samples yielded spirochaetes. Both ‘abnormal’ and ‘watery’ samples were significantly more likely to contain spirochaetes than were normal samples ($\chi^2 = 8.23; P = < 0.01$ and $\chi^2 = 20.94; P = < 0.001$, respectively). Watery samples were also significantly more likely to contain spirochaetes than were abnormal samples ($\chi^2 = 7.41; P = < 0.01$).

DISCUSSION

In this study human stool samples from seven different sources were examined. Although the time between the stool being passed and it being plated out were different for the seven sources of samples, an experiment that was conducted to assess the influence of time on viability of the spirochaetes indicated that this difference would not greatly influence apparent prevalence of colonization. Thus of 49 samples from Aboriginal children that were plated out on the day they were obtained, 47 (95.9%) still yielded viable spirochaetes when plated out again 15
days later. *Serpulina hyodysenteriae* is also known to survive for periods of up to 48 days in pig faeces stored at temperatures between 0 and 10 °C [21].

The study demonstrated that Australian Aborigines, and particularly children over 2 years of age who live in a remote rural region have a high (32.6%) prevalence of colonization with intestinal spirochaetal bacteria. Similarly, high prevalences have been reported in nationals of the Sultanate of Oman [12], in West and Central Africa [18, 22] and in Southern India [17]. In contrast to this high prevalence, in the present study only 8 of 695 stool samples (1.2%) from individuals of various ages in other parts of Western Australia and the Northern Territory were found to contain spirochaetes. It was interesting that at least 7 of these 8 positive samples came from remote regions containing large Aboriginal communities, that 6 of the samples were known to be from children, and that 2 of the 3 for which ethnic background had been recorded were Aborigines. The low apparent prevalence in these control populations could have been partially influenced by antimicrobial therapy, since individuals from all but the last (healthy) sources had been examined for gastrointestinal disturbances by doctors. Unfortunately details of any treatments administered were not recorded. Nevertheless this low prevalence in the predominantly non-Aboriginal control groups resembles the prevalence seen in a survey conducted in the United Kingdom in which only 23 of 1527 faecal samples (1.5%) from various UK nationals contained intestinal spirochaetes. That survey also had parallels with the present study in that 16 (70%) of the positive samples from the UK came from British Asians, and 12 of these 16 had visited the Indian subcontinent in the preceding year.

From these studies it appears that there may either be ethnic or environmental influences predisposing to spirochaetal colonization of the intestine. Australian Aborigines who live in remote communities resemble individuals living in developing countries in that they have poor nutrition and sanitation, and their children also frequently suffer from diarrhoea [23]. In the pig, following colonization with the spirochaete *Serpulina (Treponema) hyodysenteriae*, it is believed that aspects of the diet, including such things as selenium deficiencies [24] and the presence of certain dietary components [25] may influence the development of swine dysentery. Components of the intestinal microflora may also enhance [26] or prevent [27] proliferation of spirochaetes and influence subsequent development of swine dysentery. In the pig the proliferation of intestinal spirochaetes is uncommon before weaning; in both the present work and in a recent study in Oman [12] there was significantly more spirochaetal colonization in individuals over 2 years of age than in younger children or babies. Spirochaetes may normally proliferate after weaning as a result of changes in the environment of the large intestine following introduction of solid food, or as a result of loss of passive immunity. Our work also suggests that Aboriginal children are more commonly colonized than are adult Aborigines, suggesting the possibility of acquisition of immunity with age.

All the spirochaetes that were recovered were weakly beta-haemolytic, and the biochemical reactions of 7 of 14 Australian and 3 Italian isolates resembled those of *S. innocens* of the pig. However, half the Australian isolates produced indole, a feature which is uncommon amongst *S. innocens* isolates [28]. Six isolates were
both indole positive and had alpha-glucosidase but not alpha-galactosidase activity: these are features normally possessed by *S. hyodysenteriae* [20]. We intend to differentiate these various spirochaetes further to determine their overall diversity and their genetic relationships to the porcine spirochaetes. This will be particularly interesting for those spirochaetes that were recovered from the same children in different years. If these isolates can be shown to be identical it will demonstrate either the existence of long-term colonization or of repeated infections of individuals with a specific spirochaetal clone.

The role of spirochaetal bacteria in the production of gastrointestinal disorders in humans remains uncertain. Numerous studies have associated the presence of intestinal spirochaetes with a variety of conditions, particularly long-standing diarrhoea and rectal bleeding [9, 10, 13, 14]. Other workers have noted spirochaetal colonization in healthy individuals, and have concluded that the bacteria have no clinical significance [12, 15, 16]. In the current study most Aboriginal children were found to have abnormal stools, and many were colonized with intestinal spirochaetes. A striking aspect of the study was that spirochaetes were significantly more likely to be recovered from ‘abnormal’ and ‘watery’ samples than from normal specimens, with watery samples being significantly more likely to yield spirochaetes than ‘abnormal’ samples. These results strongly suggest that the bacteria were involved in the aetiology of the diarrhoea. However, it has also been suggested that individuals with diarrhoea are more likely to have spirochaetes in the faeces than are healthy individuals because the bacteria, which may be part of the normal microflora, are flushed from the colonic crypts during the process of diarrhoea [29]. Even though it could be demonstrated that nearly all our control populations with diarrhoea did not have spirochaetes in their faeces, this could have been either because the populations from which they were drawn do not normally have spirochaetes in their colonic crypts, or because some of them may have received antimicrobial therapy that had eliminated the spirochaetes. Our healthy control group (source 7) had not been treated with antibiotics, but then again they did not have diarrhoea. We are therefore currently unable to prove that the spirochaetes that we recovered from individuals in the Aboriginal communities were the cause of their diarrhoea.

From our own observations and from recent biochemical and DNA homology studies [10, 30], it is apparent that human intestinal spirochaetes are a heterogeneous group. By analogy with porcine intestinal spirochaetes, it seems possible that certain strains of certain genetic groups of human intestinal spirochaetes possess pathogenic potential. We hypothesize that unknown factors in the Aboriginal communities, as in many developing countries, predispose to colonization and proliferation of the spirochaetes. We suggest that within the Aboriginal communities there are certain strains of spirochaetes that have pathogenic potential, and that when these proliferate they contribute to colonic malabsorption and to diarrhoea. We are currently investigating this hypothesis further.

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