

Intestinal spirochaetes in a Gulf Arab population

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SUMMARY

Spirochaetal bacteria were isolated from the faeces of 11·4% of 1000 hospital patients and 26·7% of 292 healthy local nationals in the Muscat area of the Sultanate of Oman. A case-control study of patient records showed the isolation of the spirochaete to be associated with Omani nationality, age in excess of 2 years and attendance at self-referral health-care facilities. No association was found with symptomatic intestinal disease or microscopic abnormalities of the stool.

INTRODUCTION

The presence of spirochaetal bacteria in human faeces has been noted many times since the first report in 1884 [1]. Although precise descriptions have been hampered by the difficulties of *in vitro* cultivation, the organisms which have been described appear to represent a heterogeneous group. Most recent descriptions of spirochaetes from the lower gastrointestinal tract concern organisms of two types. One has been identified as *Brachyspira aalborgi* in some studies, a regularly coiled spirochaete of length up to 6 μm and diameter approximately 0·2 μm . The second larger type is generally described as being 4–20 μm in length and of 0·2–0·5 μm diameter with irregular coils. The latter organism was named '*Spirochaeta eurygyrata*' in early reports when direct microscopy of clinical material was the only available form of identification, although Hogue in 1922 also used the name for faecal spirochaetes cultured in liquid medium [2]. '*Spirochaeta eurygyrata*' is no longer accepted taxonomically and this organism does not have a current name. Recent reports of organisms fitting the earlier descriptions of the larger spirochaete have tended instead to use the terms 'faecal spirochaete' or 'intestinal spirochaete', and the organism has been tentatively placed in the genus *Treponema* [3]. It is a strict anaerobe and may be cultivated on media containing spectinomycin 400 $\mu\text{g}/\text{ml}$, colistin 10 $\mu\text{g}/\text{ml}$ and rifampicin 1 $\mu\text{g}/\text{ml}$ [4]. Biochemical investigation has demonstrated marked production of esterase C4, esterase C8, and β -galactosidase; it displays activity against a wide range of carbohydrates [5].

There is considerable variation in the reported prevalence of the larger spirochaete in faeces and some studies [5, 6] have suggested that it is more common in eastern and African populations than in Europeans. Although doubt has been cast on the spirochaete's ability to cause disease [5, 6], there are also reports of its recovery from patients with bowel disorders [2, 3, 7], and the organism's role as a pathogen therefore remains uncertain.

The following study was undertaken to discover the prevalence of the organism in a Gulf Arab population, and its association with disease.

MATERIALS AND METHODS

Specimens examined

During the period January to November 1988, spirochaetes were sought in 1000 faecal specimens submitted from patients at the Royal Hospital in the Sultanate of Oman. Specimens were examined at random according to the availability of selective culture medium and gas-generating kits; only those submitted for bacteriological or parasitological examination were included in the study. Routine microscopy and culture for common enteric pathogens (including *Campylobacter* sp.) were performed on each sample, and the age and sex of the patient were recorded along with details of the hospital area submitting the specimen. In the case of those specimens yielding spirochaetes, patient case-notes were examined and the patient's presenting complaint, medical speciality, nationality, and result of routine stool examination were recorded. The same information was collected for a control group which comprised those patients found negative for faecal spirochaetes and whose specimen laboratory-number immediately followed that of spirochaete-positive patient. The significance of differences between the characteristics of patient and control groups were evaluated using the χ^2 test and the Bon-Ferroni correction for multiple use of χ^2 comparisons was applied.

The prevalence of the spirochaete was also assessed in the normal Omani population; 292 normal subjects were examined, the group comprised school-children, college students, Orthopaedic and Maternity Unit inpatients, and a few hospital staff. Non-Omanis, individuals with a history of bowel disorder or recent antibiotic treatment were excluded. Specimens from this group of patients were cultured on selective medium only, microscopy and routine culture were not performed.

In order to determine the frequency of recovery of the spirochaete in comparison with recognized enteric pathogens, a record was kept of all salmonella, shigella and campylobacter isolates made in the laboratory during the period of the study.

Cultural methods

All specimens were examined using the laboratory's routine methods. These comprised direct microscopy for parasites, and inoculation of selenite F broth and of DCA, SS and MacConkey agars (Oxoid) for salmonellae and shigellae, these media were incubated overnight at 37 °C. Each specimen was cultured for *Campylobacter* sp. on columbia agar base containing 7% lysed horse blood and Skirrow's supplement (Oxoid). *Campylobacter* plates were incubated at 43 °C for 48 h in jars containing a microaerophilic gas-generating kit (Oxoid), in most cases the jars contained a *C. jejuni* positive control. Specimens were examined for faecal spirochaetes within 24 h of receipt. Blood agar base No. 2 (Oxoid) containing colistin 10 µg/ml and spectinomycin 400 µg/ml was inoculated with a small amount of material taken from the centre of each sample with a wooden applicator. Within 1 h of inoculation the plates were placed in an anaerobe culture jar and an anaerobic environment was created by use of gas-generating kits

Table 1. Characteristics of patients with faecal spirochaetes and case-controls

	Patients	Controls
Male	53	65
Female	61	49
Mean age (years)	20.9	20.7
Age range (years)	0.6–60	NB to 75
Subjects aged <2 years	2	17
Omanis*	104	82
Attending Royal Hospital	87	70
Attending other hospitals	27	44
Attending self-referral clinic	77	45
Attending other clinics	37	71
Diarrhoea†	20	33
Abdominal pain†	55	33
Vomiting†	10	14
Unexplained microscopical abnormalities in faeces‡	11	16

* Out of 106 patients and 98 controls.

† Out of 93 patients and 92 controls.

‡ White or red blood cells without a recognized pathogen.

NB, newborn.

(Oxoid). The plates were incubated at 37 °C for between 3 and 4 weeks before inspection.

Characterization of isolates

Ten morphologically typical strains were examined to establish similarity with isolates in other reports. Seven of the strains were patient isolates and three were from the control population. Biochemical activity of the organisms was investigated using the API Zym system (API, Basingstoke, UK) and the length and diameter of each strain were measured with a micrometer eyepiece.

RESULTS

Identification of the spirochaete

After 3–4 weeks' incubation the spirochaete grew on the selective medium in flat spreading colonies which were weakly β -haemolytic. Ageing cultures were composed of confluent growth with a matt metallic sheen and the presence of cystic forms on dark-ground microscopy. In agreement with other reports [5, 7], the spirochaetes produced detectable amounts of esterase C4, esterase C8 and β -galactosidase in the API Zym system, most strains showed weak positive reactions for acid and alkaline phosphatases. The mean length of the organisms was 7.9 μ m and the mean diameter 0.4 μ m.

Recovery of the spirochaete from hospital patients

The spirochaete was detected in a total of 114 (11.4%) of the 1000 faecal samples from hospital patients, the mean age of these patients was 20.9 years and 61 were female. It was not possible to locate all patient and case-control hospital records, and complete clinical information could be obtained for only 93 patients and 92 controls; for the remaining subjects, information was limited to patient

Table 2. *Age distribution of normal subjects examined for faecal spirochaetes*

	Age group (years)							
	5-9	10-14	15-19	20-24	25-29	30-34	35-39	> 39
Number examined*	34	127	61	34	16	15	3	4
Number positive	10	22	24	11	7	3	0	1
Percentage positive	29	17	39	32	44	20		

* Ages were unknown for two subjects.

identity details only. Combinations of abdominal pain, diarrhoea and vomiting were mentioned for 75 of the 93 patients and also for 75 of the 92 controls (Table 1). For 18 patients and 17 controls, clinical details appeared unrelated to enteric infection, for example trauma, chest pain, malaria, leukaemia. Nationality details were available for 106 spirochaete-positive patients and for 98 controls. All but two (both Indian) of those yielding the spirochaete were Omanis. Sixteen of the controls were of other nationality - 7 Indian, 3 European, 3 Egyptian, 2 Sudanese and 1 Syrian.

Three significant differences were found between the patient and case-control groups. Patients from whom spirochaetes were isolated were more likely to be attending a self-referral clinic ($P < 0.01$), were more likely to be Omanis ($P < 0.01$) and were more likely to be over 2 years of age ($P < 0.01$); no significant differences between patients and controls were found in mean age, sex, medical speciality, hospital attended, abdominal pain, diarrhoea, vomiting, or microscopical and cultural abnormality of the faeces.

Recovery of the spirochaete from normal subjects

The normal population group comprised 186 females and 106 males and had an average age of 15.5 years. The spirochaete was detected in 45 (24.2%) of the females and in 33 (32.0%) of the males, i.e. in 78 (26.7%) overall. The prevalence in this group for different age ranges is shown in Table 2.

During the study period, a total of 8960 faecal specimens was received by the laboratory; 148 (1.7%) yielded salmonellae (including *S. typhi*), shigellae were found in 336 (4.1%), and *Campylobacter* sp. in 5 (0.06%).

DISCUSSION

There are difficulties in comparing the faecal spirochaetes described in different studies. Early reports contained morphological descriptions only, and later biochemical characterization has sometimes indicated differences between isolates. For example, one recent study [7] found the organisms to produce esterase C4 but not acid phosphatase, whilst another report described the opposite [8]; both studies had obtained their organisms from patients with diarrhoea and the cultural and microscopic appearances of the bacteria were similar. Thus it is not possible to be certain that the organisms examined in the present work are identical with those previously described, however they do possess the morphological and biochemical attributes of the larger type of spirochaete noted in other studies [3, 5, 7], and are clearly distinct from *Brachyspira aalborgi*.

The high prevalence of the spirochaete found in the present work contrasts with the 1.5% reported by Tompkins and colleagues [5] in a study in which faeces from a predominantly European population were cultured. However the detection of the organism in a large proportion of patients accords with an early report [6] in which it was claimed that microscopical examination had revealed the spirochaete in every subject examined, a study which was also made in a mixed population in a developing country.

Few other large studies have been undertaken with the aim of assessing carriage rates of intestinal spirochaetes. In one study of a European population undergoing sigmoidoscopy, *Brachyspira aalborgi* [9] was the spirochaete detected, and was present in 5% of 300 subjects. Since the study was based upon light- and electron-microscopical examination of mucosal biopsies, it may have underestimated the prevalence of the organism. The study concluded that *Brachyspira aalborgi* played no pathogenic role. A study conducted in Rwanda [10] claimed a high prevalence of a spirochaete similar to *Treponema hyodysenteriae*, which may be the same as the organism isolated in the present work. The Rwandan study was made by culturing faeces on to medium containing spectinomycin, vancomycin and amphotericin B. The spirochaete was detected in 23% of 93 healthy subjects and, as in Oman, in a smaller proportion of hospital patients. The reasons for the lower prevalence in hospital patients are unknown. In the present study, the proportion of normal subjects yielding the spirochaete exceeded the prevalence of 11.4% in the patient group at all ages (Table 2) and it is therefore unlikely that the different mean age of the two groups accounted for the different prevalences. Similarly, the greater proportion of females in the normal group is unlikely to have led to an overestimate of spirochaete prevalence since normal Omani females yielded the organism less frequently than normal Omani males (24.2 vs. 32.0%). Possibly the organism is encountered less commonly in hospital patients because they are more likely to be receiving treatment with antibiotics to which the spirochaetes are susceptible, although in the present study medical records were insufficiently reliable to establish this. The same reason might account for the increased prevalence among patients in self-referral clinics. It is also possible that bowel disorders sufficient to result in the submission of a faecal sample disturb the microbial flora and eliminate the organism.

The frequent isolation of the spirochaete from asymptomatic Omanis in comparison with expatriates confirms observations that the spirochaete is associated with particular racial groups [5]. Differences in the type of bacteria recovered from Omani and European populations are further emphasised by the finding of *Salmonella* spp. and *Shigella* spp. in approximately 6% of faeces whilst *Campylobacter* spp. were detected in only five of almost 9000 specimens submitted for analysis.

There are many reports of the isolation of the intestinal spirochaete from small numbers of patients who might be expected to have a disturbance of the normal bowel flora. This is particularly the case in western populations where isolation has been associated with homosexuality [3, 6, 11], symptomatic intestinal disease [3, 7, 12], and a history of recent travel to the developing world [4, 13]. However, it is clearly possible that, in these circumstances, the organism represents an incidental finding rather than the cause of disease. In the present study, the lack of association of the organism with specific symptomatology or microscopic

abnormalities, coupled with its recovery from 26·7% of healthy individuals, suggest that it is a member of the normal bowel flora and that its presence is associated with ethnic rather than pathological factors. No evidence was found that it is a cause of intestinal disease.

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