Epidemiol. Infect. (1998), **121**, 219–225. Printed in the United Kingdom

Serpulina pilosicoli, waterbirds and water: potential sources of infection for humans and other animals

S. L. OXBERRY, D. J. TROTT AND D. J. HAMPSON*

Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia

(*Accepted 12 February 1998*)

SUMMARY

Serpulina pilosicoli was isolated from 8 of 43 (19%) faecal specimens obtained from feral waterbirds sampled around a small lake at Perth Zoological Gardens, Western Australia, and from 3 of 7 (43%) samples of the lake water. The organism was only isolated from 1 of 204 (0·5%) samples from captive birds and animals in the zoological collection. Multilocus enzyme electrophoresis analysis of the isolates showed that they were genetically diverse, and none had identical electrophoretic profiles as those previously obtained from human beings, dogs, pigs and other avian species. To determine the survival time of *S. pilosicoli* in water, cells of strain 1648 were seeded into lake and tap water, and incubated at 4, 25 and 37 °C. The organism could be recultured from lake water for up to 66 days at 4 °C, and for 4 days at 25 °C. A healthy human volunteer who drank water seeded with *S. pilosicoli* strain Wes B became colonized, and developed abdominal discomfort and headaches. Contamination of water by faeces may represent a source of *S. pilosicoli* infection for both humans and animals.

INTRODUCTION

The spirochaete *Serpulina pilosicoli* was recently described as the causative agent of porcine intestinal spirochaetosis (PIS), an infection of the porcine large intestine resulting in mild colitis and diarrhoea [1]. *S. pilosicoli* also infects a variety of other hosts, including human beings [2–4], dogs [5], and birds [6]. In humans, colonization of the large intestine with *S. pilosicoli* also has been termed intestinal spirochaetosis (IS), and has been associated with various gastrointestinal disorders, including chronic diarrhoea and rectal bleeding [7–9]. The organism is most commonly isolated from individuals who are immunocompromised [10], homosexual males [11], or those living in developing countries or underprivileged indigenous groups [12–14]. In infected individuals, large numbers of *S. pilosicoli* cells are often seen attached by one end to the colonic or rectal epithelium [15]. Recently more invasive cases have been described in both immunocompromised and immunocompetent individuals, with intestinal spirochaetes being present in enterocytes, goblet cells, macrophages and Schwann cells, and in some cases being associated with epithelial ulceration and necrosis, and crypt abscessation [16–18]. *S. pilosicoli* has also been isolated from the bloodstream of critically-ill patients [19].

There is evidence that *S. pilosicoli* can be transmitted between animal species. Experimentally, day-old SPF chicks and newly weaned pigs have been infected with human strains of *S. pilosicoli*, resulting in clinical signs and lesions typical of PIS/IS [20, 21]. In addition, a study conducted in Papua New Guinea using pulsed-field gel electrophoresis showed that some *S. pilosicoli* strains isolated from dogs were identical to strains from humans living in the same environment [22]. It might be suspected that pigs also could be a potential source of infection for human beings, but, despite a high prevalence in human beings

* Author for correspondence.

in the latter study, no village pigs were found to be infected [14].

Commercial poultry flocks are also frequently (28–53%) colonized by intestinal spirochaetes [23, 24], although only a small proportion of isolates have been shown to be *S. pilosicoli* [6]. In a study of feral waterbirds in Ohio, high rates of colonization also were found, with unidentified intestinal spirochaetes being observed in caecal smears from 50 of 57 (88%) birds [25]. In comparison, the carriage rate of intestinal spirochaetes in 122 caged birds belonging to 37 different species at the Ohio zoological collection was only 4·1% [26], and only 1 of these strains was found to be *S. pilosicoli* [27].

In view of the possible zoonotic potential of *S. pilosicoli*, the current study was designed to examine the prevalence of this spirochaete in wild waterbird populations. A site was chosen where these birds were living in close contact with both human beings and other potentially susceptible species. An attempt was also made to look for its presence and determine its survival time in water, and to test whether water contaminated with *S. pilosicoli* was infective to human beings. The study site was a small lake at the Perth Zoological Gardens – this was frequented by feral waterbirds, was visited daily by many human beings, and was in close proximity to large numbers of captive birds and other animals.

MATERIALS AND METHODS

Collection of faecal specimens and lake water

Freshly deposited faecal specimens from 43 feral waterbirds (predominantly ducks) were collected from the concrete rim surrounding a shallow 5000 m² lake, containing approximately 300000 kilolitres of water, at the Zoological Gardens, Perth, Western Australia. The identity of the individual bird that deposited the sample could not always be determined, although it was noted that at least 24 species frequented the lake. All faecal specimens were scooped into sterile jars, which contained 1 ml of sterile phosphate buffered saline (PBS, pH 7·2) to prevent them from drying out. Batches of 500 ml of lake water were also collected into sterile containers from each of 7 different locations around the edge of the lake. Finally, 204 fresh faecal samples were obtained from 91 captive animals (from 10 major orders) and 113 caged birds (from 15 major orders) held in the collection at the Zoological Gardens. None of these captive animals or birds had received antimicrobial agents in the previous month.

Culture conditions

Faecal samples were streaked directly onto selective Trypticase Soy agar (BBL) plates supplemented with 5% defibrinated ovine blood, 400 μg ml^{-1} spectinomycin, and 25 μg ml^{-1} each of vancomycin and colistin. These were incubated at 37 °C in anaerobic jars in an atmosphere of 94% N_2 and 6% CO_2 for 7–10 days. The presence of spirochaetes was initially identified by the appearance of a zone of weak β-haemolysis surrounding a low flat haze of bacterial growth, and was confirmed by viewing the characteristic spirochaete morphology under a phase contrast microscope. Spirochaetes were subcultured until they were in pure culture and then transferred into anaerobic, pre-reduced Trypticase Soy broth supplemented with 2% foetal bovine serum (Kunkle's medium) [28]. Lake water was centrifuged at 3836 g for 15 min and the pellet plated onto the selective agar and incubated as described above.

DNA extraction and polymerase chain reaction

DNA was extracted from primary growth on the isolation plates using a previously described culture resuspension diatomaceous earth extraction (CRDEX) protocol [29]. This was applied to plates from 17 randomly selected feral waterbird faecal specimens, all 7 of the Zoo lake water samples, and 12 of the zoo animal and caged bird samples. All spirochaetes obtained in pure culture also were subjected to CRDEX/PCR to confirm their identity as *S. pilosicoli*.

PCR tests for identifying *S. pilosicoli* were carried out as described previously [29, 30]. The reaction amplified a 1300 base pair segment of the *S. pilosicoli* 16S rRNA gene [29]. The PCR products were subjected to electrophoresis in 1·5% agarose gels in Tris-borate buffer (0·09 M Tris, 0·09 M borate, 0·02 M EDTA, pH 8·0) followed by staining in 0·5 μg ml^{-1} ethidium bromide, and were viewed and photographed over ultraviolet light.

Spirochaete survival in water

All strains used were obtained from the collection held at the Reference Centre for Intestinal Spirochaetes at

https://doi.org/10.1017/S0950268898008863 Published online by Cambridge University Press

Murdoch University. Porcine *S. pilosicoli* strain 1648 and *S. hyodysenteriae* strain WA 15 were grown to mid-log phase in Kunkle's broth, centrifuged at 1087 ***g*** for 10 min, resuspended in sterile distilled water, recentrifuged and resuspended in sterile distilled water. Cell numbers were counted using a haemocytometer with a phase contrast microscope, and each strain was seeded in duplicate into aliquots of Perth Zoo lake water (not containing *S. pilosicoli*, as determined by culture), or Perth tap water, to make the following concentrations: 10^7, 10^6, 10^5 and 10^4 spirochaetes ml^{-1} of water. Aliquots of 1 ml of each concentration were placed at 4, 25 and 37 °C. Every 2–5 days 50 μl of seeded water was plated onto selective agar and incubated as previously described. Subsequently *S. pilosicoli* strain Wes B, isolated from an Aboriginal child [13], and isolate P1, recovered from the lake water, were similarly inoculated into lake water at a cell concentration of 10^7 ml^{-1} and held at 4 °C.

Experimental infection of a healthy human being

This experiment, which involved a human volunteer, was conducted with the advice of the Murdoch University Human Ethics and Biosafety Committees. *S. pilosicoli* strain Wes B was grown to mid-log phase in Kunkle's broth, then centrifuged at 1087 ***g*** for 10 min. The cells were washed once in PBS and counted. An average of $2{\cdot}9 \times 10^9$ freshly prepared spirochaetes were added to 100 ml of tap water on each of three successive days, and drunk by one of us (D.J.H.), a healthy 43-year-old male. Faecal swabs were taken on days 1, 3, 5–9, 14, 34, 41, 48, 50, 52, 55, 58 and 70 post-ingestion (pi), and cultured on the selective agar. Unclotted blood samples (10 ml) were taken on days 34, 41, 48 and 50 pi. These were added directly to 100 ml hemoline anaerobic blood culture medium (BioMerieux), and cultured and 37 °C for 2 weeks. Samples were examined under a phase-contrast microscope, and subcultured to Trypticase Soy agar containing 5% blood but no antibiotics, and incubated under the same conditions as described previously. The volunteer then took 400 mg metronidazole (Flagyl, Rhone-Poulenc) twice daily for 5 days, commencing on day 52 pi.

Multilocus enzyme electrophoresis (MLEE)

The methodology used for preparing the cells, buffers and enzymes and the electrophoretic running conditions used for analysing the *S. pilosicoli* isolates have been published previously [31, 32]. Fifteen enzymes were tested: acid phosphatase, alcohol dehydrogenase, hexokinase, alkaline phosphatase, arginine phosphokinase, glutamate dehydrogenase, phosphoglucose isomerase, guanine deaminase, mannose-6-phosphate isomerase, fructose-1,6-diphosphatase, esterase, phosphoglucomutase, peptidase, nucleoside phosphorylase and superoxide dismutase. Distinctive mobility variants for each enzyme were interpreted as reflecting different alleles at the structural locus encoding that enzyme. Allele profiles for the 15 enzymes were recorded as electrophoretic types (ETs). Genetic distances between ETs were calculated as the proportion of fixed allelic loci at which dissimilar alleles occurred. The ETs were compared with those previously obtained in our laboratory for a variety of intestinal spirochaetes, including those obtained for over 200 *S. pilosicoli* isolates from human beings, pigs, dogs and birds [6, 27, 31, 32].

RESULTS

Culture, PCR and MLEE examination of spirochaete isolates from bird faecal specimens and lake water

Spirochaetes were observed on 32 of the 43 plate cultures (74·4%) from the feral waterbirds, although only 20 isolates (46·5%) were obtained in pure culture. Of these, 8 (18·6% of the total faecal samples, and 40% of the total isolates) were shown to be *S. pilosicoli* using the CRDEX/PCR protocol. MLEE also grouped these isolates with other strains of *S. pilosicoli*. The 8 isolates belonged to different ETs, and they all varied from each other in up to 9 alleles. None shared the same ET with isolates that we have previously analysed from humans, pigs, dogs and other avian species [6, 27, 31, 32]. Of the 17 samples from birds that were subjected to CRDEX/PCR off the primary plate, 8 (47%) gave positive signals, whilst *S. pilosicoli* was only successfully isolated from 2 of these samples.

Weakly β haemolytic intestinal spirochaetes were isolated from 3 of the 7 (43%) samples of lake water, although direct CRDEX/PCR detected *S. pilosicoli* DNA in the material harvested from cultures from an additional sample. The isolated spirochaetes were shown to be *S. pilosicoli* by CRDEX/PCR and by MLEE analysis. The three isolates belonged to different ETs from the isolates from the waterbirds, and were different from each other, however two were

https://doi.org/10.1017/S0950268898008863 Published online by Cambridge University Press

Table 1. *The survival time in days of* Serpulina pilosicoli *strain 1648 cells suspended in lake and tap water at various concentrations, and maintained at 4 or 25 °C. The procedure was done in duplicate, and results for both test are shown where different results were obtained*

	Concentration of cells ml^{-1}			
	10^4	10^5	10^6	10^7
Lake water				
4 °C	12	12–26	12–33	40–66
25 °C	< 1	1	3	4
Tap water				
4 °C	< 1	< 1	< 1–3	< 1–7
25 °C	< 1	< 1	1	1

only separated from each other by allelic differences at two loci.

Zoological collection

Twelve of the 204 (6%) samples from captive animals and caged birds in the zoological collection contained spirochaetes, but only a single isolate, which was obtained from a domesticated pig in the children's zoo corner, was found to be *S. pilosicoli*. CRDEX/PCR did not detect any additional *S. pilosicoli* infections. This isolate belonged to a different ET to the isolates obtained from the feral waterbirds and lake water.

Spirochaete survival in water

All concentrations of the *S. hyodysenteriae* cells held at 25 and 37 °C, and the three lower cell concentrations held at 4 °C remained viable for less then 1 day in both lake and tap water. The spirochaetes at the 10^7 cell concentration remained viable for 7 days in tap water and for 14 days in lake water. The cells of *S. pilosicoli* strain 1648 also survived for longer in lake water than in tap water, and for longer at the higher cell concentrations (Table 1). Generally they survived for much longer than the *S. hyodysenteriae* strain, although none of the cell concentrations in either lake or tap water survived for longer than 1 day at 37 °C. The longest survival time for this strain was 66 days in lake water at 4 °C, with an initial cell concentration of 10^7 ml^{-1}. At 25 °C the same cell concentration only survived for 4 days. The other two *S. pilosicoli* strains, Wes B and P1 respectively survived 70 and 28 days at concentrations of 10^7 ml^{-1} in lake water at 4 °C.

Experimental infection

No spirochaetes were isolated from any of the faecal samples collected from the volunteer over the period 1–14 days pi. Around 30 days pi, the volunteer started to develop mild nausea and abdominal discomfort, including a feeling of abdominal bloating. Diarrhoea did not occur. Cultures of swabs taken on days 34 through 52 pi all contained a dense pure growth of spirochaetes to the last streak made on the plate. These spirochaetes were confirmed as *S. pilosicoli* by CRDEX/PCR, and they were shown to have the same ET as the inoculated strain (Wes B). No spirochaetes were isolated from the blood culture bottles. Starting at around 46 days post-ingestion, the volunteer also started to develop headaches, which progressively became more severe. Therapy was instigated on day 52, and by day 55 and on all subsequent days no spirochaetes could be isolated. The volunteer ceased to have any symptoms by the end of the therapy.

DISCUSSION

The lake at the Perth Zoological Gardens attracts up to 2000 wild waterbirds at any one time, the majority of which are transitory and only come to the lake to feed, however a few species nest at the site. Many of these birds were colonized with intestinal spirochaetes, with cultures from 32 of 43 (74·4%) of the faecal samples showing microscopic evidence of spirochaetes. Spirochaetes were only successfully isolated from 20 (46·5%) samples. This high colonization rate was not dissimilar to the rates found in Western Australian poultry flocks using the same culture medium [24]. In that study 10–90% of samples in 53·3% of broiler breeder flocks and 35·1% of layer flocks contained spirochaetes [24]. A distinct difference however was the fact that no *S. pilosicoli* isolates were found in the poultry, whereas 8 of the 20 waterbird isolates obtained here belonged to this species. Furthermore, use of the CRDEX/PCR method on the primary plates suggested that up to 47% of the samples contained *S. pilosicoli*. The identities of the other waterbird isolates that were obtained in pure culture were not established, although the results of MLEE suggested that they belonged to a variety of other species in the genus *Serpulina*.

The high population density of waterbirds on the lake was likely to have facilitated the spread of

https://doi.org/10.1017/S0950268898008863 Published online by Cambridge University Press

infection amongst them. It was not possible to identify individual infected birds, nor to correlate colonization with clinical signs in the birds. The *S. pilosicoli* isolates showed a high degree of genetic diversity, and there was no evidence for a single clone of the organism being highly represented. Wild waterbirds apparently may act as a reservoir of *S. pilosicoli* infection, with recycling of infection perhaps occurring through ingestion of contaminated water. Consistent with this hypothesis, 3 of the 7 (43%) lake water samples contained *S. pilosicoli* cells which could be recovered by culture, and CRDEX/PCR suggested a fourth sample also contained the organism. Presumably these spirochaetes were deposited into the water in waterbird faeces – even though none of the three isolates was identical to any of the waterbird isolates obtained. This may simply reflect the great diversity found amongst the isolates obtained, and the relatively small number of isolates analysed. Another possible explanation for the presence of the organisms in the water could be that *S. pilosicoli* is a normal long-term inhabitant of fresh water, particularly shallow water containing a lot of organic matter. This important possibility is supported by the prolonged survival time of the three different strains of organism in experimentally-seeded lake water kept at cooler temperatures. Clearly this deserves further investigation, but in any case it is likely that the lake water could have served as a reservoir of infection or reinfection for the waterbirds that frequented it.

Although the *S. pilosicoli* strains isolated from the waterbirds and the lake water were different from those we have previously isolated from pigs, dogs, poultry and human beings, this does not exclude the possibility that the strains are capable of cross-species transmission. Interestingly, of the captive animals and birds in the zoological collection, only a single pig was colonized with an *S. pilosicoli* isolate, and this animal originated from a local piggery with a previous history of PIS [33]. Similarly low colonization rates have been reported amongst animals and birds in the Ohio zoo [26]. The high colonization rate amongst the feral waterbirds therefore apparently did not represent a significant health risk to other animal species at the zoo, perhaps because there was no direct contact between the feral birds and the captive species. In contrast, the lake shore was a popular picnic site for families visiting the zoo, and the possibility that individuals could become infected with *S. pilosicoli* strains that were present in high numbers in that concentrated area requires further investigation.

This study concentrated on *S. pilosicoli*, however a number of other uncharacterized spirochaetes also were detected in the birds. Multilocus enzyme electrophoresis studies have previously shown that there are at least 3 species of intestinal spirochaete that are pathogenic to birds, including *S. pilosicoli*, *Serpulina intermedia* and *Serpulina alvinipulli* [6, 34, 35]. Further studies are required to determine whether the uncharacterized isolates from the feral birds included strains of these other species that are pathogenic to commercial poultry.

To our knowledge this is the first report of intestinal spirochaetes being isolated from a water source. The confirmed presence of *S. pilosicoli* in lake water has potential implications for both animal and public health, particularly where unchlorinated water supplies are used – for example in certain pig or poultry farms, or by human beings living in developing communities. We have previously speculated on the potential role of different water supplies as an explanation for different prevalence of *S. pilosicoli* infections amongst villages in the Highlands of Papua New Guinea [14].

When testing the survival time of *S. pilosicoli* in water, we included a strain of the porcine pathogen *S. hyodysenteriae* as a control. Its relatively short survival time in water (maximum of 14 days in lake water at 4 °C) was consistent with previous reports [36]. In contrast the *S. pilosicoli* strains survived for considerably longer, up to 70 days in lake water held at 4 °C. Not unexpectedly, survival time was diminished at higher temperatures, with lower starting concentrations of cells, and in metropolitan tap water compared to lake water. Generally *S. pilosicoli* was more resistant to adverse environmental conditions than was *S. hyodysenteriae*, and its enhanced survival improves its chances of transmission to susceptible hosts.

In human beings, infection with *S. pilosicoli* is most common in immunocompromised individuals, in homosexual males, and in people in certain developing communities. Whilst this unusual distribution of infection may reflect relative opportunities for transmission of the organism, and the ability to mount a protective immune response against it, we have speculated that ethnic or dietary differences may influence susceptibility [13, 14]. To investigate this further, one of us, who lacked any of these putative predisposing factors, drank cultures of the organism suspended in tap water. The strain used (Wes B) was one that had been shown to survive for prolonged

periods in water, had originated from an Aboriginal child with diarrhoea [13], and had colonized and induced intestinal disease in experimentally infected chicks and pigs [20, 21]. The volunteer became heavily colonized by the strain, thus demonstrating that simple exposure to large numbers of the organism can induce colonization regardless of ethnic origin, diet or immune status of the individual. Unfortunately it was uncertain whether end-on attachment of the spirochaete to the intestinal epithelium occurred, as intestinal biopsies were not taken.

Since the study only involved one individual, the results can only be regarded as preliminary, but nevertheless several interesting findings were obtained. Firstly, there was a surprisingly long delay between exposure and a level of intestinal colonization that was detectable by faecal culture. This presumably reflects either the poor sensitivity of the isolation medium and culture conditions used, or that the organism may have had difficulty in overcoming factors which resist colonization in the large intestine. Similarly, in experimentally infected pigs we occasionally experience a prolonged incubation time before faecal excretion and development of clinical signs associated with PIS occur [37]. Secondly, the volunteer experienced a range of vague gastrointestinal disturbances approximately 1 month pi, and it was this that prompted him to culture his faeces again at 34 days pi. It is likely that similar mild but disturbing symptoms would go undiagnosed without the availability of specialized diagnostic tests and facilities. Thirdly, colonization at this time was very heavy, and continued for a considerable period until therapy was instigated. In our studies in Papua New Guinea we calculated that individuals are colonized by a single strain of *S. pilosicoli* for an average of approximately 4 months [14]. This ability to persist demonstrates that the spirochaete is a highly successful parasite. Fourthly, the fact that the individual developed severe headaches, which ceased after the spirochaete was removed using metronidazole, is of interest. It was uncertain whether the headaches were connected to the infection, and it would be useful to conduct experimental infections in more volunteers to study this possibility. Previously we have demonstrated the presence of *S. pilosicoli* in the bloodstream of debilitated patients [19], and although we did not isolate the spirochaete from the bloodstream of the volunteer in this study, we do not exclude the possibility that the spirochaete underwent a transient bacteraemia.

Transmission of *S. pilosicoli* in water is a new concern for both human and animal health. It is uncertain whether the strains of the organism we found in water or infecting the feral waterbirds can infect humans or other species of animal, but cross-species transmission of the organism previously has been demonstrated. In any case, it is likely that contamination of drinking or swimming water with faeces containing human strains of *S. pilosicoli* could result in human infection, and that this colonization may have significant long-term effects on health.

ACKNOWLEDGEMENTS

We thank the staff at the Perth Zoological Gardens for their help in collecting samples, Roslyn Atyeo for her advice on the use of the PCR protocol, and Julie Ardley and Sandra McPhail for their technical assistance.

REFERENCES

1. Trott DJ, Stanton TB, Jensen NS, Duhamel GE, Johnson JL, Hampson DJ. *Serpulina pilosicoli* sp. nov.: the agent of porcine intestinal spirochetosis. Int J System Bacteriol 1996; **46**: 206–15.
2. Trott DJ, Stanton TA, Jensen NS, Hampson DJ. Phenotypic characteristics of *Serpulina pilosicoli* the agent of intestinal spirochaetosis. FEMS Microbiol Lett 1996; **142**: 209–14.
3. Stanton TB, Trott DJ, Lee JI, McLaren AJ, Hampson DJ, Paster BJ, Jensen NS. Differentiation of intestinal spirochaetes by multilocus enzyme electrophoresis analysis and 16S rRNA sequence comparisons. FEMS Microbiol Lett 1996; **136**: 181–6.
4. Atyeo RF, Oxberry SL, Hampson DJ. Pulsed-field gel electrophoresis for the sub-specific differentiation of *Serpulina pilosicoli* (formerly '*Anguillina coli*'). FEMS Microbiol Lett 1996; **141**: 77–81.
5. Duhamel GE, Muniappa N, Mathiesen MR, et al. Certain canine weakly B-hemolytic intestinal spirochetes are phenotypically and genomically related to spirochetes associated with human and porcine intestinal spirochetosis. J Clin Microbiol 1995; **33**: 2212–5.
6. McLaren AJ, Trott DJ, Swayne DE, Oxberry SL, Hampson DJ. Genetic and phenotypic characterization of intestinal spirochetes colonizing chickens and allocation of known pathogenic isolates to three distinct genetic groups. J Clin Microbiol 1997; **35**: 412–7.
7. Harland WA, Lee FD. Intestinal spirochaetosis. B M J 1967; **3**: 718–9.
8. Gad A, Willen R, Furugard K, Fors B, Hradsky M. Intestinal spirochaetosis as a cause of longstanding diarrhoea. Uppsala J Med Sci 1977; **82**: 49–54.

9. Douglas JG, Crucioli V. Spirochaetosis: a remediable cause of diarrhoea and rectal bleeding? B M J 1981; **283**: 1362.
10. Kasborher S, Gelderblom HR, Arasteh K, et al. Intestinale spirochatose bei HIV-infektion. Dtsch med Wschr **115**: 1499–506.
11. Law CLH, Grierson JM, Stevens SMB. Rectal spirochaetosis in homosexual men: the association with sexual practices, HIV infection and enteric flora. Genitourin Med 1944; **70**: 26–9.
12. Barrett SP. Intestinal spirochaetes in a Gulf Arab population. Epidemiol Infect **104**: 261–6.
13. Lee JI, Hampson DJ. Intestinal spirochaetes colonizing aborigines from communities in the remote north of Western Australia. Epidemiol Infect 1992; **109**: 133–41.
14. Trott DJ, Combs BG, Mikosza ASJ, et al. The prevalence of *Serpulina pilosicoli* in humans and domestic animals in the Eastern Highlands of Papua New Guinea. Epidemiol Infect 1997; **119**: 369–79.
15. Trivett-Moore NL, Gilbert GL, Law CLH, Trott DJ, Hampson DJ. Isolation of *Serpulina pilosicoli* from rectal biopsies showing evidence of intestinal spirochetosis. J Clin Microbiol 1998; **36**: 261–5.
16. Kostman JR, Patel M, Catalano E, Camacho J, Hoffpauir J, DiNubile MJ. Invasive colitis and hepatitis due to previously uncharacterised spirochetes in patients with advanced immunodeficiency virus infection. Clin Infect Dis. 1995; **21**: 1159–65.
17. Guccion JG, Bentor DA, Zeller J, Termanini B, Saini N. Intestinal spirochaetosis and acquired immunodeficiency syndrome: ultrastructural studies of two cases. Ultrastruct Pathol 1995; **19**: 15–22.
18. Padmanabhan V, Dahlstrom J, Maxwell L, Kaye G, Clarke A, Barrett PJ. Invasive intestinal spirochaetosis: a report of three cases. Pathol 1996; **28**: 62–7.
19. Trott DJ, Jensen NS, Saint Girons I, et al. Identification and characterisation of *Serpulina pilosicoli* isolates recovered from the blood of critically-ill patients. J Clin Microbiol 1997; **35**: 482–5.
20. Trott DJ, McLaren AJ, Hampson DJ. Pathogenicity of human and porcine intestinal spirochetes in day-old specific pathogen free chicks: an animal model of intestinal spirochetosis. Infect Imm 1995; **63**: 3705–3710.
21. Trott DJ, Huxtable CR, Hampson DJ. Infection of newly-weaned pigs with human and porcine strains of *Serpulina pilosicoli*. Infect Imm 1996; **64**: 4648–54.
22. Trott DJ, Mikosza ASJ, Combs BG, Oxberry SL, Hampson DJ. Population genetic analysis of *Serpulina pilosicoli* and its molecular epidemiology in villages in the Eastern Highlands of Papua New Guinea. Int J System Bacteriol 1998; In press.
23. Dwars RM, Smit HF, Davelaar FG, Van't Veer W. Incidence of spirochaetal infections in cases of intestinal disorder in chickens. Avian Pathol 1989; **18**: 591–5.
24. McLaren AJ, Hampson DJ, Plant SL. The prevelance of intestinal spirochaetes in commercial poultry flocks in Western Australia. Aust Vet J 1996; **74**: 31–3.
25. Swayne DE, McLaren AJ. Avian intestinal spirochaetes and intestinal spirochaetosis. In: Hampson DJ, Stanton TB, eds. Intestinal spirochaetes in domestic animals and humans. Wallingford, UK: CAB International, 1997; 267–300.
26. Stoutenburg JE, Swayne DE, Hoepf TM, Wack R, Kramer L. Frequency of intestinal spirochetes in birds, rats, and pigs from a zoologic collection and private rhea farm in Ohio. J Zoo Wildlife Med 1995; **26**: 272–8.
27. Trott DJ, Atyeo RF, Lee JI, Swayne DE, Stoutenburg JW, Hampson DJ. Genetic relatedness amongst intestinal spirochaetes isolated from rats and birds. Lett Appl Microbiol 1996; **23**: 431–6.
28. Kunkle RA, Harris DL, Kinyon JM. Autoclaved liquid medium for propogation of *Treponema hyodysenteriae*. J Clin Microbiol 1986; **24**: 669–71.
29. Park NY, Chung CY, McLaren AJ, Atyeo RF, Hampson DJ. Polymerase chain reaction for the identification of human and porcine spirochaetes recovered from cases of intestinal spirochaetosis. FEMS Microbiol Lett 1995; **125**: 225–30.
30. Atyeo RF, Hampson DJ. Diagnosis of swine dysentery and intestinal spirochaetosis by the use of polymerase chain reaction tests on faecal samples. In: Hennessy DP, Cranwell PD, eds. Manipulating pig production V. Weribee, Victoria, Australia: Australasian Pig Science Association, 1995; 186.
31. Lee JI, Hampson DJ, Lymbery AJ, Harders SJ. The porcine intestinal spirochaetes: identification of new genetic groups. Vet Microbiol 1993; **34**: 273–85.
32. Lee JI, Hampson DJ. Genetic characterisation of intestinal spirochaetes, and their association with disease. J Med Microbiol 1994; **40**: 365–71.
33. Atyeo RF, Trott DJ, Robertson ID, Buddle JR, Hampson DJ. Epidemiological analysis of *Serpulina pilosicoli* sp. nov. within a high-health status herd. Proc 14th Int Pig Vet Soc Cong 1996, Bologna, Italy, 287.
34. Stanton TB, Fournie-Amazouz E, Postic D, et al. Recognition of two new species of intestinal spirochetes: *Serpulina intermedia* sp. nov. and *Serpulina murdochii* sp. nov. Int J System Bacteriol **47**; 1007–12.
35. Stanton TB, Postic D, Jensen NS. *Serpulina alvinipulli*, sp. nov., a new *Serpulina* species enteropathogenic for chickens. Int J System Bacteriol 1998; In press.
36. Chia SP, Taylor DJ. Factors effecting the survival time of *Treponema hyodysenteriae* in dysenteric faeces. Vet Rec 1978; **103**: 68–70.
37. Hampson DJ, Trott DJ. Intestinal spirochaete infections of pigs: an overview with an Australian perspective. In: Hennessy DP, Cranwell PD, eds. Manipulating Pig Production V. Weribee, Victoria, Australia: Australasian Pig Science Association, 1995; 139–69.