Epidemiol. Infect. (1997), **119**, 369–379. Printed in the United Kingdom © 1997 Cambridge University Press

The prevalence of *Serpulina pilosicoli* in humans and domestic animals in the Eastern Highlands of Papua New Guinea

D. J. TROTT[1], B. G. COMBS[2], A. S. J. MIKOSZA[1], S. L. OXBERRY[1], I. D. ROBERTSON[1], M. PASSEY[2], J. TAIME[2], R. SEHUKO[2], M. P. ALPERS[2] AND D. J. HAMPSON[1]*

[1] *Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch WA Australia 6150*
[2] *PNG Institute of Medical Research, Goroka, Eastern Highlands Province, Papua New Guinea*

(*Accepted 11 June 1997*)

SUMMARY

In a survey of five villages in the Eastern Highlands of Papua New Guinea, *Serpulina pilosicoli* was isolated from rectal swabs from 113 of 496 individuals (22·8%). Colonization rates ranged from 22·6–30·1% in four of the villages but was only 8·6% in the other village. In comparison colonization was demonstrated in only 5 of 54 indigenous people (9·3%) and none of 76 non-indigenous people living in an urban environment in the same region. Colonization did not relate to reported occurrence of diarrhoea, age, sex, or length of time resident in a village. A second set of 94 faecal specimens was collected from 1 village 6 weeks after the first set. *S. pilosicoli* was isolated from 27 of 29 individuals (93·1%) who were positive on the first sampling and from 7 of 65 individuals (10·8%) who previously were negative. In this case, isolates were significantly more common in watery stools than in normal stools. The annual incidence of infection in the village was calculated as 93·6%, with an average duration of infection of 117 days. *S. pilosicoli* could not be isolated from any village pig ($n = 126$) despite its confirmed presence in 17 of 50 commercial pigs (34·0%) sampled at a local piggery. Four of 76 village dogs (5·3%) and 1 of 2 village ducks were colonized with *S. pilosicoli*, suggesting the possibility of cross transmission between humans and animals.

INTRODUCTION

Serpulina pilosicoli is the agent of porcine intestinal spirochaetosis (PIS), a diarrhoeal disease of commercial pigs which results in reduced weight gain and decreased feed conversion [1]. *S. pilosicoli* has a wide host distribution and also has been isolated from dogs [2], commercial poultry [3], wild birds [4], non-human primates [5] and humans [6], often in association with diarrhoea and lesions consistent with PIS. In humans the condition has been termed human intestinal spirochaetosis (HIS). The clinical significance of HIS is unclear. Some consider it to be a cause of chronic diarrhoea and rectal bleeding [7–14], and others consider it lacks clinical significance [15, 16]. In many of these studies, the aetiological agent was never cultured or was presumed to be *Brachyspira aalborgi*, a species of intestinal spirochaete that has only been isolated from humans on one occasion, and is presumed to be non-pathogenic [16, 17]. Recently it has been demonstrated that spirochaetes in some of these studies were *S. pilosicoli* [6, 18]. Furthermore, the potential significance of *S. pilosicoli* as a human pathogen has been highlighted by its recent isolation from the bloodstream of debilitated patients [19]. Human *S. pilosicoli* strains also have been shown to cause diarrhoea and histological lesions when orally inoculated into day-old chicks [20] and newly-weaned pigs [21]. This suggests that cross-species transmission

* Author for correspondence.

https://doi.org/10.1017/S0950268897008194 Published online by Cambridge University Press

of *S. pilosicoli* also may occur naturally. Multilocus enzyme electrophoresis has been used to show that human and animal strains of *S. pilosicoli* were genetically closely related, but did not share the same haplotype [6]. In previous studies, high rates of colonization (> 30%) with *S. pilosicoli* have been found in subjects from developing countries [22], disadvantaged indigenous groups [14], AIDS patients [23], and homosexual males [6, 24, 25], whilst the organisms are rarely isolated from other individuals in Western communities [14, 26].

Papua New Guinea (PNG) is a developing country where the majority of people live as subsistence farmers in villages and keep small numbers of animals, including pigs, dogs, and chickens. In the Highlands, pigs are the predominant livestock and have both economic and ceremonial significance. Piglets are often raised inside the family dwelling, and then continue to maintain close contact with humans throughout their life. In the present study, we conducted a prevalence survey amongst villagers and their animals living in the Eastern Highlands of PNG. The purposes of the study were to determine whether *S. pilosicoli* was present in Highland villagers and their animals, and to compare the rates of infection with those obtained from control populations in the Eastern Highlands (comprising humans living in an urban environment and intensively reared pigs).

MATERIALS AND METHODS

Study design and collection of specimens from humans

All experimental procedures had the prior approval of the National Ethical Clearance Committee of Papua New Guinea. Data and faecal specimens were collected from villagers and animals living in 5 villages in the Eastern Highlands Province. Villages 1 (population 203), 2 (population 162), 3 (population 136), and 4 (population 106) were located within a 20 km zone to the north west of Goroka, the capital of the Eastern Highlands Province. Village 5 (total population not determined) was situated in Okapa approximately 75 km south from Goroka (Fig. 1). Two visits were made to each village prior to collection to explain the project to the villagers and to ask for permission to obtain samples. All individuals were invited to take part in the study. At the time of collection, each individual was interviewed by a local nurse and their name, age, sex, number of family members, whether they were born in the village or had recently settled from another region, and whether they felt unwell or had diarrhoea was recorded. The type of illness reported was placed into one of five categories: diarrhoea, abdominal discomfort without diarrhoea, musculoskeletal, respiratory, and miscellaneous conditions. As poor compliance was experienced collecting faecal specimens in specimen jars left at each village overnight for this purpose, rectal swabs (Lancet Scientific and Surgical Supplies, Perth, Western Australia) were distributed to villagers and used to obtain faecal specimens on the day of each visit. Each fresh swab was immediately placed in Carey-Blair transport medium (Oxoid Ltd, Basingstoke, UK) kept at 4 °C, and cultured within 4 h. A total of 496 faecal specimens from humans were obtained from individuals in villages 1–5 (Table 1).

To relate colonisation to the appearance of the faeces, compare sampling techniques, and estimate the incidence and duration of infection, a second set of 123 faecal specimens was obtained directly in sterile specimen jars from people in village 1, 6 weeks after the first set of isolations. Faecal consistency was recorded as either normal (well formed cigar shaped), abnormal (faeces with the consistency of wet clay) or watery (loose watery diarrhoea). During this visit, 94 of the 123 samples (76·4%) were obtained from individuals who provided a sample on the first visit. Data from the 29 new individuals who did not previously provide a sample on the first visit were not included in the initial prevalence study. The 44 individuals who were culture positive for intestinal spirochaetes on the first sampling were then treated with 500 mg of tinidazole (adult dose) (Fasigyn, Pfizer Inc., New York, USA) three times daily for 3 days. Tinidazole was used in preference to metronidazole due to supply limitations. Additional faecal samples were obtained from 18 of these individuals 2 weeks after treatment. All culture positive individuals in the remaining villages also were treated with tinidazole but follow up samples were not obtained from them.

Faecal specimens in sterile specimen jars were obtained from a control population of 130 individuals, consisting of indigenous workers from the Institute for Medical Research (IMR) in Goroka, and their families living in Goroka ($n = 54$), non-indigenous members of a missionary station situated approximately 20 kg east from Goroka ($n = 65$), and non-indigenous children attending the Goroka International School ($n = 11$). Specimens in specimen jars also were obtained from 50 pigs (10 weaners, 10

https://doi.org/10.1017/S0950268897008194 Published online by Cambridge University Press

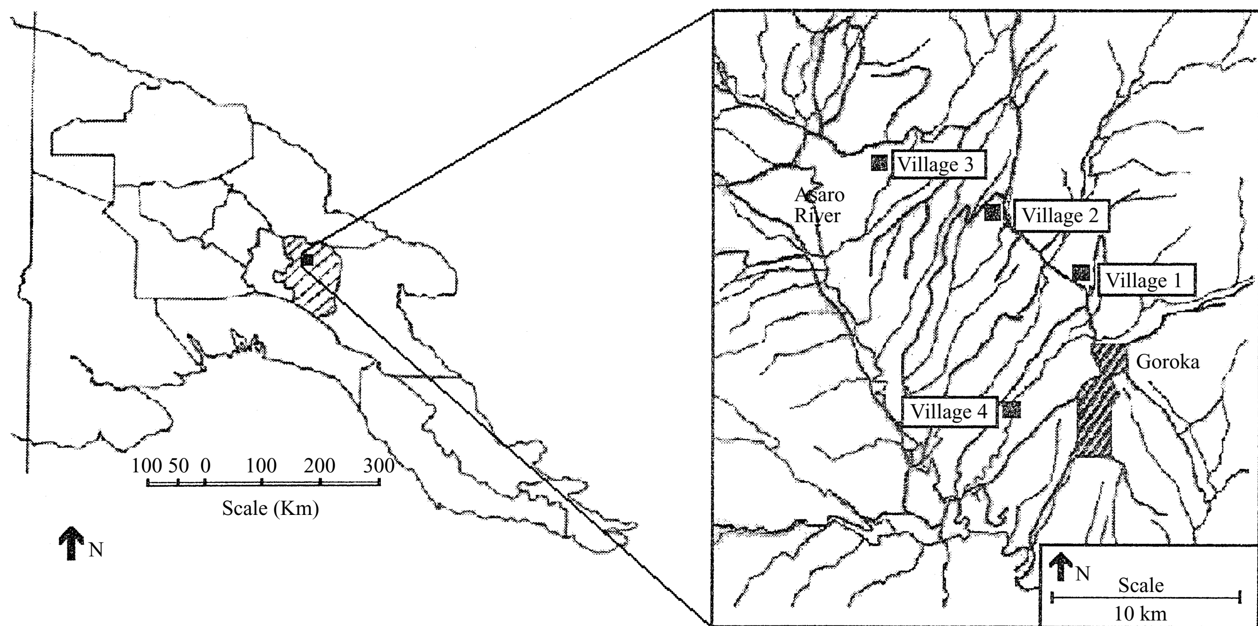

Fig. 1. Study area within the Eastern Highlands Province of Papua New Guinea. The location of the villages with respect to the town of Goroka are shown in the inset.

Table 1. *Colonization rate of* S. pilosicoli *in 496 villagers living in 5 villages in the Eastern Highlands of Papua New Guinea and 130 individuals living in the urban environment of Goroka, divided into five age categories*

	Age category (years)											
	0–2		3–5		6–10		11–18		> 18		Total	
Source	+	−	+	−	+	−	+	−	+	−	+	−†
Villages												
1	0 (0)‡	10	2 (15·4)	11	7 (28·0)	18	10 (52·6)	9	25 (31·6)	54	44 (30·1)	102
2	1 (16·7)	5	6 (42·9)	8	6 (40·0)	9	11 (33·3)	22	10 (20·0)	40	34 (28·8)	84
3	0 (0)	6	2 (9·5)	19	2 (9·1)	20	2 (9·5)	19	5 (8·6)	53	11 (8·6)§	117
4	0 (0)	2	2 (33·3)	4	3 (25·0)	9	2 (50·0)	2	5 (17·2)	24	12 (22·6)	41
5	3 (23·1)	10	2 (14·2)	12	5 (45·4)	6	1 (16·7)	5	1 (14·3)	6	12 (23·5)	39
Total	4 (10·8)	33	14 (20·5)	54	23 (27·1)	62	26 (31·3)	57	46 (20·6)	177	113 (22·8)¶	383
Urban*												
Total	0 (0)	4	1 (8·3)	11	0 (0)	21	1 (3·6)	27	3 (4·6)	62	5 (3·8)‖	125

* Samples included 54 indigenous workers at the Papua New Guinea Institute of Medical Research and their families, 65 members of a Goroka based mission, and 11 children attending the Goroka International School.

† +, colonized with *S. pilosicoli*; −, not colonized with *S. pilosicoli*.

‡ Numbers in parentheses indicates percentage of individuals colonized with *S. pilosicoli*.

§ The prevalence in village 3 was significantly lower than in the other four villages ($\chi^2 = 21{\cdot}59$, $P < 0{\cdot}001$).

‖ Villagers were significantly more likely to be colonized with intestinal spirochaetes than the combined control population ($\chi^2 = 22{\cdot}92$, $P < 0{\cdot}001$).

https://doi.org/10.1017/S0950268897008194 Published online by Cambridge University Press

Table 2. *Recovery of intestinal spirochaetes from village animals and commercial pigs in the Eastern Highlands of Papua New Guinea*

	Animal type				
Location	Dogs	Pigs‖	Pigs§	Birds	Rats
Village 1					
Total	27	29	NT‡	2	9
Number positive	3 (1)*	0		0	1
Per cent positive	11·1 (3·7)	0		0	11·1 (†)
Village 2					
Total	21	45	NT	5	6
Number positive	2 (1)	0		0	0
Per cent positive	9·5 (4·8)	0		0	0
Village 3					
Total	18	42	NT	11	3
Number positive	0	0		2	0
Per cent positive	0	0		18·2 (9·1)	0
Village 4					
Total	10	10	NT	NT	NT
Number positive	2 (2)	0			
Per cent positive	20 (20)	0			
Piggery					
Total	NT	NT	50	NT	NT
Number positive			21 (17)		
Per cent positive			42 (34)		
Total					
Total	76	126	50	18	18
Number positive	7 (4)	0	21 (17)	2 (1)	1 (†)
Per cent positive	9·2 (5·3)	0	42 (34)	11·1 (5·6)	5·6 (†)

* Numbers in parentheses refer to the specific recovery of *S. pilosicoli*.
† The identity of the single rat isolate could not be confirmed.
‡ Not tested.
§ Commercial pigs.
‖ Village pigs.

finishers and 30 growers) from an intensive piggery located approximately 25 km south west of Goroka.

Collection of specimens from animals

For collection of faecal specimens from animals, the species, owner, clinical appearance and, where it could be directly observed, the consistency of the faeces of each animal, were recorded. These samples were categorized into normal, abnormal and watery, as described previously for human stools from village 1. Rectal swabs were obtained and placed in transport medium as described above. Rats also were trapped in villages 1–3, and specimens for culture were obtained directly from the caecum. A total of 126 specimens were obtained from village pigs, 76 from village dogs, 14 from village chickens, 18 from trapped rats, and 2 samples each from domestic ducks and pigeons (Table 2).

Culture conditions

The specimens were plated onto Trypticase Soy agar (Becton Dickinson, Cockeysville, Maryland, USA) supplemented with 5% bovine blood, 400 μg/ml spectinomycin and 25 μg/ml each of colistin and vancomycin (TSB-CVS agar), and incubated in anaerobic jars in an atmosphere of 94% N_2 and 6% CO_2 at 39 °C for 5–15 days. The presence of intestinal spirochaetes was confirmed by the appearance of weak β-haemolysis around individual bacterial colonies, and examination of wet preparations under a phase contrast microscope for typical spirochaete cells. Growth from plates that were positive for intestinal spirochaetes was subcultured to fresh plates to obtain the spirochaetes in pure culture. If significant contamination with other bacteria was present, growth was plated onto TSB-CVS agar supplemented with 5 μg/ml polymixin B and 25 μg/ml nalidixic

https://doi.org/10.1017/S0950268897008194 Published online by Cambridge University Press

acid. All antibiotics used in selective culture were obtained from Sigma Chemical Company, St Louis, Missouri. Bacterial colonies from pure plates were subcultured at least three times prior to inoculation into 7 ml of Kunkle's broth medium [27] and incubated at 37 °C on a rocking platform for 72–96 h, or until mid log phase growth was achieved. Pure cultures were transferred to 250 ml of Kunkle's medium and again incubated at 37 °C on a rocking platform for 72–96 h. Aliquots from mid log phase cultures (10^8–10^9 cells ml^{-1}) [1] were preserved at −80 °C and the reminder was centrifuged (10000 g, 4 °C, 20 min), washed in sterile phosphate buffered saline, and centrifuged again (10000 g, 4 °C, 20 min).

Polymerase chain reaction for *S. pilosicoli*

The protocol utilized, including primers and reaction conditions, was for a hot start two phase reaction previously determined and optimised in our laboratory for the identification of *S. pilosicoli* [28]. The enzyme and primers were held in separate phases until the commencement of the heating cycle (10 °C) by a layer of 25 μl of Chill-out wax (MJ Research, Watertown, Massachusetts, USA). The PCR produced a 1330 base pair 16S ribosomal DNA gene product using one primer based upon a unique region of *S. pilosicoli* 16S rDNA and one universal 16S rDNA sequencing primer. The technique was modified by the delivery of target DNA to the top phase of the reaction. A wooden toothpick was dipped once into the bacterial pellet obtained after centrifugation of the culture and briefly touched once onto the top layer of the PCR reaction. Care was taken not to pick up too many cells which may have inhibited the PCR reaction. For each isolate, three areas of the pellet were individually sampled from and placed into three separate PCR reactions. Each batch of PCR amplifications included DNA from positive (*S. pilosicoli* strain P43/6/78^T) and negative (*S. hyodysenteriae* strain B78^T) control cultures.

Statistical analysis

The statistical significance of differences between groups was assessed using Yates corrected χ^2 test. Odds ratios were determined for selected groups where statistical significance was demonstrated using the χ^2 test [29]. Two way analysis of variance was used to assess statistical significance between the mean age of infected versus uninfected individuals. Cohen's Kappa statistic was used to determine the degree of agreement between the first and second sampling methods (swabs on first sampling, specimen jars on second sampling) for isolating spirochaetes from individuals in village 1.

RESULTS

Recovery of spirochaetes from humans

In the initial prevalence study spirochaetes were cultured from 113 of the 496 humans living in the 5 villages (22·7%). In contrast only 5 of the 130 samples from the control populations were positive (3·8%). All 5 positive individuals were either PNG Institute of Medical Research workers or family members, and the prevalence amongst this subset of the control population (indigenous people living in an urban environment) was 9·3% (Table 1). Overall, villagers were significantly more likely to be colonized with intestinal spirochaetes than both the combined control population ($\chi^2 = 22{\cdot}92$, $P < 0{\cdot}001$) and the IMR control group alone ($\chi^2 = 4{\cdot}51$, $P < 0{\cdot}05$). Calculated positivity rates were based on the number of patients sampled, rather than the entire village population.

No significant difference was demonstrated between the mean age of infected (20·7 years) and uninfected individuals (19·3 years) living in villages. Neither the sex of the individual (62 of 262 males and 51 of 234 females positive for *S. pilosicoli*), whether they were a recent settler in the village (10 of 57 settlers and 103 of 439 villagers positive for *S. pilosicoli*), nor whether the individual reported illness (Table 3), including diarrhoea or abdominal pain, were significantly associated with colonization with intestinal spirochaetes. Colonization rates for children that were 18 years or under (24·5%) were not significantly different from those of adults (20·6%). When village children were divided into 4 age categories: 2 years and under (before the age of weaning), 3–5 (pre-school), 6–10 (pre-adolescent), and 11–18 (adolescent), those in the 2 years and under age group had the lowest rate of colonization (10·8%), however no one group was significantly different from another (Table 1).

The prevalence of colonization with intestinal spirochaetes at the time of sampling for villages 1–5 was 30·1%, 28·8%, 8·6%, 22·6% and 23·5% respectively. The prevalence in village 3 was significantly lower than in the other four villages ($\chi^2 = 21{\cdot}59$, $P < 0{\cdot}001$), and was even lower than the prevalence

https://doi.org/10.1017/S0950268897008194 Published online by Cambridge University Press

Table 3. *Recovery of* S. pilosicoli *from villagers divided into health categories 1–6*

Category	Type	+	−†	Positive (%)
1	Diarrhoea	2	23	8
2	Abdominal pain	12	42	22·2
3	Respiratory	17	40	29·8
4	Musculoskeletal	11	21	33·3
5	Other*	2	4	33·3
6	Healthy	69	314	18·0
Total		113	383	22·7

* Category 5 included two individuals with typhoid, one with dysentery, 1 with poor weight gain, 1 with an eye infection and one with a sexually transmitted disease.
† +, colonized with *S. pilosicoli*; −, not colonized with *S. pilosicoli*.

amongst the IMR control group (9·3%), although not significantly so. Using odds ratios, individuals in villages 1, 2, 4, and 5 were 4·6, 4·3, 3·1, and 3·3 times respectively more likely to be colonized with intestinal spirochaetes than individuals in village 3.

The overall rate of colonization at the second sampling in village 1 was again 30·1% (37 of 123 individuals tested). Twenty-nine of the sample were from individuals who had not previously been tested (23·6%), and 3 of these individuals (10·3%) were positive. Twenty-seven of the 29 individuals who were culture positive on the first sampling were also culture positive on the second sampling. Seven of the other 65 individuals who were negative on the first sampling were positive on the second sampling. An individual was significantly more likely to be colonized on the second sampling if they were positive on the first sampling ($\chi^2 = 58{\cdot}88$ $P < 0{\cdot}001$; odds ratio: individuals positive on the first sampling were 111·9 times more likely to be positive on the second sampling than were those who were negative on the first sampling). The Kappa statistic was greater than 0·75 (0·786), indicating excellent agreement between the two sampling procedures in relation to categories of colonization.

Stools from the second sampling in village 1 were graded into three categories, normal, abnormal and watery (Table 4). Watery samples were significantly more likely to contain intestinal spirochaetes than were normal samples ($\chi^2 = 6{\cdot}46$, $P < 0{\cdot}05$), but no other association was significant. All watery samples were obtained from individuals who were positive at both collections. Two weeks after treatment with tinidazole, only 2 of 18 colonized individuals were still colonised.

Table 4. *Recovery of* S. pilosicoli *from 94 individuals in village 1 using faecal sample pots to determine faecal consistency*

Faecal consistency	+	−*	Positive (%)
Normal† $n = 53$	15	38	28·3
Abnormal‡ $n = 31$	12	19	32·2
Watery‖ $n = 10$	7	3	70·0
Total $n = 94$§	34	60	36·1

* +, colonized with *S. pilosicoli*; −, not colonized with *S. pilosicoli*.
† Normal: cigar shaped well formed stool.
‡ Abnormal: unformed, wet clay-like or greasy stool.
§ Data from 29 individuals who gave samples for the first time in sample pots not included.
‖ Watery: loose and watery (diarrhoea).

Recovery of spirochaetes from animals

Intestinal spirochaetes were isolated from 7 of 76 village dogs (9·2%), comprising 3 of 27 dogs from village 1, 2 of 21 dogs from village 2, and 2 of 10 dogs from village 4. Spirochaetes were isolated from 1 of 14 village chickens, 1 of 18 rats, and 1 of 2 ducks, but not from any of the 126 village pigs (Table 2). In contrast spirochaetes were isolated from 6 of 10 weaner pigs, 14 of 30 grower pigs, and 1 of 10 finisher pigs at the commercial piggery (Tables 2, 6). Forty of the 50 samples (80%) obtained from the piggery were categorized as normal and 18 of these (45%) contained intestinal spirochaetes. Five of the samples were abnormal (two samples containing intestinal spirochaetes) and 5 were watery (one sample containing spirochaetes) (Table 5). No significant association was demonstrated between colonisation of commercial pigs with intestinal spirochaetes and faecal consistency. Of the 76 faecal specimens from dogs, 34 were normal, 34 had an abnormal consistency, 7 were watery and 1 was from a dog with dysentery (Table 5). Intestinal spirochaetes were isolated from 2 dogs with normal faeces, 1 dog with abnormal faeces, 2 dogs (puppies from the same litter) with watery faeces and the dog with dysentery.

Identification of *S. pilosicoli*

All except 6 of the 171 intestinal spirochaete strains obtained in the study that were tested gave positive

https://doi.org/10.1017/S0950268897008194 Published online by Cambridge University Press

Table 5. *Recovery of intestinal spirochaetes from the faeces of village dogs and commercial pigs divided into 4 categories based upon the consistency of the faeces*

	Faecal consistency				
Animal	Normal*	Wet clay†	Watery‡	Dysentery‖	Total
Village dogs	34 (2§)	34 (1)	7 (2)	1 (1)	76 (7)
Weaners	10 (6)	—	—	—	10 (6)
Growers	24 (12)	3 (1)	3 (1)	—	30 (14)
Finishers	6 (0)	2 (1)	2 (0)	—	10 (1)
Pigs (total)	40 (18)	5 (2)	5 (1)	—	50 (21)

* Normal: cigar shaped well formed stool.
† Abnormal: unformed, wet clay-like or greasy stool.
‡ Watery: lose and watery (diarrhoea).
§ Numbers in parentheses indicate the number of samples in each category that contained intestinal spirochaetes.
‖ Dysentery: bloody diarrhoea.

reactions in the *S. pilosicoli* PCR. All of the human strains tested, 17 strains from the piggery, 4 of 7 dog strains, and the single duck strain were confirmed as *S. pilosicoli*. The *S. pilosicoli* strains isolated from dogs were obtained from villages 1 and 2 (1 strain each from dogs with normal faeces and dysenteric faeces respectively), and village 4 (2 strains from puppies with watery diarrhoea). The identity of the intestinal spirochaete isolated from a rat could not be verified as it became overgrown with contaminating organisms and died after several subcultures.

DISCUSSION

Relatively few studies have used culture techniques to examine the prevalence of intestinal spirochaete infection in developing communities [14, 22]. These previous studies were conducted prior to the classification of *S. pilosicoli* as a new species, and no attempt was made at the time to identify the intestinal spirochaetes isolated. Recently strains from these studies in Oman and in an Aboriginal community in Western Australia, as well as strains from Italy, New South Wales, the United Kingdom, and the United States have been confirmed as *S. pilosicoli* [6, 18]. The present study conducted in PNG is the first large scale attempt to specifically determine the prevalence of *S. pilosicoli* in humans and animals living in close proximity. The methodology for culture in the present study was identical to that used in the study conducted in Western Australia [14], and only differed from the study conducted in Oman [22] in the type of plates used to propagate the spirochaetes. Research in our laboratory suggests that the level of detection of culture for intestinal spirochaetes ranges from 10^3–10^5 organisms per gram of faeces (R. F. Atyeo and D. J. Hampson, unpublished data). Therefore, some of the individuals who were culture negative in the present study may have been colonized but were shedding intestinal spirochaetes below the level of detection. It is assumed that the presence of *S. pilosicoli* in the faeces reflects attachment of the organisms to the epithelium of the large intestine. In a previous study, in a developing country, 9 of 14 rectal biopsies obtained from volunteers in Southern India were shown to have intestinal spirochaetes intimately attached to the epithelium, however culture and identification of the organisms was not attempted [30].

The present study demonstrated that *S. pilosicoli* is endemic in villagers in the Highlands of PNG, with rates of colonization (8·6–30·1%) comparable to those found in Oman (22·7%) and amongst Aboriginal people in the remote north west of Western Australia (32·6%). However it must be mentioned that only approximately half of the people residing in each village were sampled, therefore estimates of the colonization rate are based upon individuals sampled rather than the total population. In agreement with previous studies, low rates of colonization were found in urbanized indigenous (9·3%) and non-indigenous (0%) individuals [14, 26]. A similarity between the current study and the studies conducted in Oman and Australia was the comparatively low rate of colonization amongst children aged 2 years and under. Although age did not significantly influence the

https://doi.org/10.1017/S0950268897008194 Published online by Cambridge University Press

colonization rate in the present study ($\chi^2 = 8{\cdot}12$, $P < 0{\cdot}1$), children 2 years and under had the lowest rate of colonization (10·8%), and those children who were colonized with *S. pilosicoli* were already in their second year. Two years was chosen as the cut-off point for the first age group, as the majority of village children in Papua New Guinea are not fully weaned until this age. The low rate of colonization in humans less than 2 years is analogous to the situation with intestinal spirochaetal infections in pigs, where both *S. pilosicoli* and *S. hyodysenteriae* are pathogens of the post weaning-period [31].

Factors which predispose to the high rate of colonization in developing communities when compared to other populations have not been determined, but may include poor hygiene, lowered immune status associated with poor nutrition, and differences in the type of diet compared to modern Western diets [14]. The nutritional status of the villagers in Papua New Guinea was observed to be adequate, although bordering on protein deficiency, however hygiene standards in the villages were low due to poor housing, and inadequate sanitation facilities, running water, and electricity. This could clearly facilitate transmission by the faecal-oral route. Many villages in the region obtained their water from the various branches of the Asaro river. As the environmental conditions in each of the villages were very similar, including population density and housing structure, it was difficult to provide reasons for the low rate of colonisation in village 3 when compared to the other villages. One explanation was that individuals in village 3 had access to water with less faecal contamination. *S. pilosicoli* has been isolated from lake water frequented by ducks colonized with the organism (S. L. Oxberry and D. J. Hampson, unpublished data), but it has not yet been determined whether *S. pilosicoli* is present in river water in our study location in PNG.

The colonization rate in village 3 was almost identical to that recorded in indigenous people living in the urban environment of Goroka. Goroka has a chlorinated water supply and connected sewerage system, thus limiting these avenues as possible sources of infection for the inhabitants, A total of 26 of the 54 people sampled who lived in Goroka (48·1%) reported visiting relations living in villages on weekends, however only 2 of these individuals were colonized with *S. pilosicoli*. Another possible source of infection for town dwellers was interaction with villagers at local markets.

Colonization with *S. pilosicoli* was not associated with any particular gastrointestinal symptom reported. Individuals who reported acute diarrhoea at the time of sampling had the lowest rate of colonization amongst the five disease categories. Aboriginal children with watery or abnormal faeces previously have been shown to have a significantly higher rate of colonization with *S. pilosicoli* than those with normal faeces [14]. In the second sampling from village one, faecal specimens were obtained in jars so that they could be visually examined and graded into three categories to give some correlation with the study conducted in Australian Aborigines. Whilst watery faeces were significantly more likely to contain *S. pilosicoli* than normal faeces, the association was not strong, and a large number of individuals with normal faeces were still colonized with the organism. Nevertheless, the suggestion that *S. pilosicoli* can cause disease in humans is supported by infection studies in animals where strains isolated from humans, pigs and dogs have been shown to attach by one cell end to the colonic epithelium and cause microvillous effacement and cytoskeletal changes in day-old chicks [20, 32], and crypt abscesses in newly weaned pigs [21]. In the present study no attempt was made to obtain colonic rectal biopsies to determine whether *S. pilosicoli* was attached to the epithelium of the large intestine of infected individuals. It remains uncertain whether *S. pilosicoli* is a commensal, an opportunist, or a frank enteric pathogen in humans, as it appears to be in other species.

Using the Kappa statistic, excellent agreement was demonstrated between the two sampling methods (rectal swabs and specimen jars) used to obtain faecal specimens from individuals in village 1, suggesting that there was little difference in the sensitivity of the two sampling techniques. As 27 of the 29 individuals were still colonized on the second sampling, most infections appeared to last for at least 6 weeks. Seven out of 65 individuals who were culture negative on the first sampling became positive at some stage during the 6 week interval, representing a rate of new infections over the 42 day period of 10·8%. This estimate assumes that the collection of faecal specimens in sample pots did not significantly improve the recovery of intestinal spirochaetes. When converted to a per annum rate, it was calculated that approximately 93·6% of the village population would become infected at some stage during a given year. The inclusion of 29 samples obtained in specimen jars from individuals who were not sampled on the first

https://doi.org/10.1017/S0950268897008194 Published online by Cambridge University Press

occasion gave a second sampling prevalence in village 1 of 30·1%. This was identical to the overall village prevalence obtained 6 weeks earlier. As the prevalence was stable, and given the incidence rate over this 42 day period was 10·8%, on average, infection in an individual would last approximately 117 days [29]. These estimates assume that a culture positive individual was colonized with a single strain of the bacterium, and reinfection did not occur over the 6-week period.

Studies using genetic fingerprinting techniques are currently being conducted to determine relationships between the *S. pilosicoli* strains isolated during the study. This will confirm the extent and nature of genetic diversity within the *S. pilosicoli* population, and whether human and animal strains share the same haplotype. *S. pilosicoli* has previously been shown to be genetically diverse, however the same strain of bacteria has been isolated on two separate occasions from an Aboriginal child with chronic diarrhoea over an interval of 1 year, and single strains have colonized groups of 3–10 children in the same study [6, 14]. Further studies are required to examine local immune responses to *S. pilosicoli* in chronically colonized individuals.

A surprising feature of the study was the lack of colonization of village pigs with *S. pilosicoli*, especially when compared to the high prevalence amongst commercial pigs from a local piggery, and the fact that other animal species in the villages were colonized (although at a lower prevalence). Village pigs and dogs had every opportunity to be infected since they scavenged through the villages and the surrounding bush, where they had free access to human faeces containing the organism. European breeds of pigs are known to be susceptible to strains of *S. pilosicoli* isolated from humans [21], hence there may be some genetic influences on colonization. Village pigs belonged to a distinct subspecies, *Sus scrofa papuensis*, whereas the infected commercial pigs in this study were the typical European Large White-Landrace cross. Environmental and dietary factors also may be involved. Diet has been shown to influence colonization of pigs with the related spirochaete *Serpulina hyodysenteriae* [33, 34], and the same may apply to *S. pilosicoli*. Village pigs predominantly scavenge, or are fed raw sweet potato, whereas the commercial pigs were fed a balanced mash feed based on cereals and legumes. Pigs raised in a low population density outdoor environment also may be more resistant or less exposed to infection than intensively raised livestock. This has been shown in previous studies in villages in the region where the carriage of *Streptococcus suis* type 2 [35] and rotavirus [36] by village pigs was found to be low. This was attributed to the non-intensive husbandry procedures adopted in the villages.

Seven of 76 village dogs (9·2%) were colonized with intestinal spirochaetes, and 4 of these strains (5·3%) were identified as *S. pilosicoli*. The prevalence of intestinal spirochaetes in PNG village dogs was lower than the prevalence (18·7%) determined in a previous study conducted in Australia amongst dogs from 4 independent sources, including two Aboriginal communities [37]. In that study however only 1 of 38 strains isolated was shown to be *S. pilosicoli* (J. I. Lee and D. J. Hampson, personal communication). Using multilocus enzyme electrophoresis, the non-*S. pilosicoli* strains isolated from dogs (3) and pigs (3) were closely related to members of the non-pathogenic porcine species *S. innocens* (D. J. Trott and D. J. Hampson, unpublished data), suggesting that they have no clinical significance [2]. The single strain from a chicken corresponded to the recently proposed species, *Serpulina intermedia* [38]. The finding of *S. pilosicoli* strains in dogs and a duck suggests that cycling of infection between animals and humans may occur in the villages. The higher prevalence in humans than in animals suggests that humans are more likely to be a source of infection for animals than vice-versa. Intestinal spirochaetes isolated from humans and dogs have previously been shown to be genetically similar, and in some cases to have identical restriction fragment length polymorphism patterns [39]. The relationships between the animal and human strains obtained during the study are currently being determined.

This study has demonstrated that *S. pilosicoli* is endemic in humans living in villages in the Highlands of Papua New Guinea, with an average prevalence of 22·8%. Annual incidence approached 100% and infection of an individual was calculated to last approximately 4 months. A small proportion of animals in the villages were infected, suggesting that the main cycle of infection was from human to human. Whilst the study did not demonstrate any particular association between colonization and clinical signs observed at the time of sampling, apart from watery diarrhoea in a small subset of individuals, long term cohort studies examining colonization with *S. pilosicoli* in conjunction with other bacterial and parasitological causes of diarrhoea are required to determine the clinical significance of this bacterium.

https://doi.org/10.1017/S0950268897008194 Published online by Cambridge University Press

Infection may be associated with reduced growth rates in children, as has been observed in chicks colonised with human strains of *S. pilosicoli* [20]. It has previously been suggested that *S. pilosicoli* may have synergistic relationships with gastrointestinal protozoa such as *Giardia duodenalis* [40] and *Balantidium coli* [21]. Perhaps significantly, *S. pilosicoli* has recently been isolated from the blood of seven severely-ill patients in France and the United States [19]. In view of the high rates of intestinal carriage, and the common occurrence of debilitating diseases such as hepatitis and typhoid, amongst individuals living in villages, we hypothesize that *S. pilosicoli* spirochaetemia also may occur commonly in villages in the Highlands of PNG. Studies are currently underway to examine this possibility.

ACKNOWLEDGEMENTS

DJT is in receipt of an Australian Pig Research and Development Corporation postgraduate scholarship. This study was supported by grants from Murdoch University and the Australian Research Council. We especially thank Ivan Aeno for help in collecting samples from the villages and Julie Ardley for technical assistance throughout the isolation phase. We also thank the people of the Eastern Highlands of Papua New Guinea for taking part in the study.

REFERENCES

1. Trott DJ, Stanton TB, Jensen NS, Duhamel GE, Johnson JL, Hampson DJ. *Serpulina pilosicoli* sp. no.: the agent of porcine intestinal spirochetosis. Int J System Bacteriol 1996; **46**: 206–15.
2. Duhamel GE, Muniappa N, Mathieson MR, et al. Certain canine weakly beta-hemolytic spirochetes are phenotypically and genotypically related to spirochetes associated with human and porcine intestinal spirochetosis. J Clin Microbiol 1995; **33**: 2212–15.
3. 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.
4. Trott DJ, Atyeo RF, Lee JI, Swayne DA, Stoutenburg JW, Hampson DJ. Genetic relatedness amongst intestinal spirochaetes isolated from rats and birds. Lett Appl Microbiol 1996; **23**: 431–6.
5. Duhamel GE, Elder RO, Muniappa N, Mathiesen MR, Wong VJ, Tarara RP. Colonic spirochetal infections of non-human primates associated with *Brachyspira aalborgi*, *Serpulina pilosicoli* and unclassified flagellated bacteria. In: Proceedings of the 77th Annual Meeting of the Conference of Research Workers in Animal Diseases, Chicago, 1996. Abst 89.
6. Lee JI, Hampson DJ. Genetic characterisation of intestinal spirochaetes and their association with disease. J Med Microbiol 1994; **40**: 365–71.
7. Cotton DWK, Kirkham N, Hicks DA. Rectal spirochaetosis. Brit J Ven Dis 1984; **60**: 106–9.
8. Crucioli V, Busuttil A. Human intestinal spirochaetosis. J Gastroenterol 1981; **70**: 177–9.
9. Douglas JG, Crucioli V. Spirochaetosis: a remediable cause of diarrhoea and rectal bleeding? BMJ 1981; **283**: 1362.
10. Gad A, Willén R, Furugård K, Fors B, Hradsky M. Intestinal spirochaetosis as a cause of longstanding diarrhoea. Upps J Med Sci 1977; **82**: 49–54.
11. Gebbers JO, Ferguson DJP, Mason C, Kelly P, Jewell DP. Spirochaetosis of the human rectum associated with an intraepithelial mast cell and IgE plasma cell response. Gut 1987; **28**: 588–93.
12. Harland WA, Lee FD. Intestinal spirochaetosis. BMJ 1967; **3**: 718–9.
13. Kaplan LR, Takeuchi A. Purulent rectal discharge associated with a nontreponemal spirochete. JAMA 1979; **241**: 52–3.
14. Lee JI, Hampson DJ. Intestinal spirochaetes colonising Aborigines from communities in the remote north of Western Australia. Epidemiol Infect 1992; **109**: 133–41.
15. Lee FD, Kraszweski A, Gordon J, Howie GR, McSeveney D, Harland WA. Intestinal spirochaetosis. Gut 1971; **12**: 126–33.
16. Nielsen RH, Orholm M, Pedersen JO, Hovind-Hougen K, Teglbjaerg PS, Thaysen EH. Colorectal spirochaetosis: clinical significance of the infection. Gastroenterol 1983; **85**: 62–7.
17. Hovind-Hougen K, Birch-Andersen A, Henrik-Nielsen R, et al. Intestinal spirochetosis: morphological characterization and cultivation of the spirochete *Brachyspira aalborgi* gen. nov., spec. nov. J Clin Microbiol 1982; **16**: 1127–36.
18. 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.
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 spirochaetes in day-old specific pathogen free chicks: an animal model of intestinal spirochetosis. Infect Immun 1995; **63**: 3705–10.
21. Trott DJ, Huxtable CR, Hampson DJ. Infection of newly weaned pigs with human and porcine strains of *Serpulina pilosicoli*. Infect Immun 1996; **64**: 4648–54.
22. Barrett SP. Intestinal spirochaetes in a Gulf Arab population. Epidemiol Infect 1990; **104**: 261–6.

https://doi.org/10.1017/S0950268897008194 Published online by Cambridge University Press

23. Kasbohrer A, Gelderblom HR, Arasteh K, et al. Intestinale Spirochätose bei HIV-Infektion-Vorkommen, Isolierung und Morphologie der Spirochäten. Dtsch med Wschr 1990; **115**: 1499–506.
24. Tompkins DS, Waugh MA, Cooke EM. Isolation of intestinal spirochaetes from homosexuals. J Clin Pathol 1981; **34**: 1385–7.
25. Law CLH, Grierson JM, Stevens SMB. Rectal spirochaetosis in homosexual men: the association with sexual practices, HIV infection and enteric flora. Genitourin Med 1994; **70**: 26–9.
26. Tompkins DS, Foulkes SJ, Godwin PGR, West AP. Isolation and characterisation of intestinal spirochaetes. J Clin Pathol 1986; **39**: 535–41.
27. Kunkle RA, Harris DL, Kinyon JM. Autoclaved liquid medium for propagation of *Treponema hyodysenteriae*. J Clin Microbiol 1986; **24**: 669–71.
28. Park NY, Chung CY, McLaren AJ, Atyeo RF, Hampson DJ. Polymerase chain reaction for identification of human and porcine spirochaetes recovered from cases of intestinal spirochaetosis. FEMS Microbiol Lett 1995; **125**: 225–30.
29. Martin SW, Meek AH, Willeberg P. Veterinary epidemiology principles and methods. Ames: Iowa State University Press. 1987.
30. Mathan MM, Mathan VI. Rectal mucosal morphologic abnormalities in normal subjects in Southern India: a tropical colonopathy? Gut 1985; **26**: 710–7.
31. Hampson DJ, Trott DJ. Intestinal spirochaetal infections of pigs: an overview and an Australian perspective. In: Hennessy DP, Cranwell PD, eds. Manipulating pig production V. Canberra: Australasian Pig Science Association, 1995: 139–69.
32. Muniappa N, Duhamel GE, Mathiesen MR, Bargar TW. Light microscopic and ultrastructural changes in the ceca of chicks inoculated with human and canine *Serpulina pilosicoli*. Vet Pathol 1996; **33**: 542–50.
33. Siba PM, Pethick DW, Hampson DJ. Pigs experimentally infected with *Serpulina hyodysenteriae* can be protected from developing swine dysentery by feeding them a highly digestible diet. Epidemiol Infect 1996; **116**: 207–16.
34. Pluske JR, Siba PM, Pethick DW, Durmic Z, Mullan BP, Hampson DJ. The incidence of swine dysentery in pigs can be reduced by feeding substrates that limit the amount of fermentable substrate entering the large intestine. J Nutrit 1996; **126**: 2920–33.
35. Paterson RA, Robertson ID, Sanders RC, Siba PM, Clegg A, Hampson DJ. The carriage of *Streptococcus suis* type 2 by pigs in Papua New Guinea. Epidemiol Infect 1993; **110**: 71–8.
36. Alpers D, Sanders RC, Hampson DJ. Rotavirus excretion by village pigs in Papua New Guinea. Aust Vet J 1991; **68**: 65–7.
37. Lee JI, Hampson DJ. The prevalence of intestinal spirochaetes in dogs. Aust Vet J 1996; **74**: 25–6.
38. Stanton TB, Bournié-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 1997; In press.
39. Koopman MBH, Käsbohrer A, Beckmann G, van der Zeijst BAM, Kusters JG. Genetic similarity of intestinal spirochetes from humans and various animal species. J Clin Microbiol 1993; **31**: 711–6.
40. Duhamel GE, Hunsaker BD, Mathieson MR, Moxley RA. Intestinal spirochetosis and giardiasis in a Beagle pup with diarrhoea. Vet Pathol 1996; **33**: 360–2.

https://doi.org/10.1017/S0950268897008194 Published online by Cambridge University Press