Human Intestinal Spirochetes Are Distinct from Serpulina hyodysenteriae

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Twenty-nine intestinal spirochetes isolated from Australian aboriginal children and six strains from Italian adults (HRM1, -2, -4, -5, -7, and -14) were genetically examined at 15 enzyme loci by using multilocus enzyme electrophoresis. Results were compared with those previously obtained for 188 porcine intestinal spirochetes. DNA from human strain HRM7 and porcine strain *Serpulina hyodysenteriae* P18A were also radioactively labeled and hybridized with DNA from 12 other human and porcine intestinal spirochetes. Both the multilocus enzyme electrophoresis and hybridization techniques demonstrated that the human spirochetes were not *S. hyodysenteriae*. They belonged to another distinct genetic group of spirochetes that included P43/6/78, the bacterium recovered from the first recorded case of porcine intestinal spirochetosis. Bacteria in this distinct group also differed from *Serpulina* spp. in possessing only four, five, or occasionally six axial filaments, being slightly thinner, and having more pointed ends. These findings add further weight to the possibility that human intestinal spirochetes may act as enteric pathogens.

The large intestines of Africans, Australian aborigines, Gulf Arabs, Indians, and homosexual men in Western societies are commonly colonized by anaerobic spirochetes (1, 11, 15, 18, 19, 24). In swine, intestinal spirochetes resembling those seen in humans were originally divided into two species. Of these, *Serpulina hyodysenteriae* is strongly beta-hemolytic and is the etiologic agent of a colonic infection of major economic importance, swine dysentery (8, 28). *Serpulina innocens*, although closely resembling *S. hyodysenteriae*, is weakly beta-hemolytic and is generally considered to be nonpathogenic (12, 28).

The clinical significance of colonization by intestinal spirochetes in humans is unclear. The infection has been called intestinal spirochetosis, and, in cases in which it has been diagnosed, the bacteria are found as a thick film firmly attached end-on to the colonic epithelium (14). This finding is not a feature of swine dysentery caused by infection with *S. hyodysenteriae* but does occur in another distinct condition of swine, also called intestinal spirochetosis (10, 29). The condition in swine results in soft porridge-like diarrhea, reduced food intake, and depressed growth rate and is associated with infection by weakly beta-hemolytic spirochetes. Until recently, these spirochetes were assumed to be atypical virulent strains of *S. innocens*, but we have recently demonstrated that they actually belong to a distinct genetic group of spirochetes (16).

Recent DNA homology studies have been used to suggest that human intestinal spirochetes, which are all weakly beta-hemolytic, are closely related to porcine spirochetes of the genus *Serpulina* and, moreover, belong to the pathogenic species *S. hyodysenteriae* (2, 6). In view of the clinical and histological similarities between intestinal spirochetosis in humans and that in swine and our demonstration that the spirochetes causing the condition in swine are not *S. innocens* or *S. hyodysenteriae*, we have used multilocus enzyme electrophoresis (MEE) and DNA homology determinations

to reexamine genetic relationships between intestinal spirochetes from the two species.

MATERIALS AND METHODS

Spirochetes. The 35 human spirochetal isolates that were analyzed in the present work were from the feces of patients with intestinal disorders. Twenty-nine were from Australian aboriginal children with diarrhea (15); the other six (HRM1, -2, -4, -5, -7, and -14) were from adults in Italy (26) (from A. Sanna, Rome, Italy). Results from MEE of these isolates were compared with those for 188 porcine spirochetes, details of which are given elsewhere (16).

Selected human spirochetes were characterized on the basis of their strength of beta-hemolysis, indole production, enzyme profiles in API-ZYM, and morphology under the electron microscope, as previously described for porcine spirochetes (16). The spirochetes were all propagated in prereduced anaerobic Trypticase soy broth supplemented with 2% fetal bovine serum and a 1% ethanolic cholesterol solution (13).

MEE. The methodology used for preparing cells for MEE and for analyzing the electrophoretic mobility of their constitutive enzymes followed that used previously for intestinal spirochetes from swine (16, 17). Briefly, spirochetes in late log phase were harvested from broth culture by centrifugation, washed, resuspended to 10^{11} cells in 2 ml of sonication buffer, and lysed by sonication, and the supernatant containing constitutive enzymes was harvested by centrifugation and stored at $-70°C$.

The cell lysates were subsequently thawed and electrophoresed in horizontal starch gels. The mobilities of the following 15 enzymes were assayed: acid phosphatase, alcohol dehydrogenase, adenylate kinase, alkaline phosphatase, esterase, fructose-1,6-diphosphatase, glucose phosphate isomerase, guanine deaminase, glutamate dehydrogenase, hexokinase, mannose-6-phosphate isomerase, nucleoside phosphorylase, L-leucyl-glycylglycine peptidase, phosphoglucomutase, and superoxide dismutase. Details of sub-

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strates and buffers for these enzymes are given elsewhere (27).

Enzyme banding patterns were consistent with a single locus coding for each enzyme, and mobility variants of the enzymes were interpreted as the products of different alleles at the corresponding locus. Groups of one or more isolates with the same alleles at all loci were referred to as being an electrophoretic type (ET). Genetic diversity (h) at each enzyme locus was calculated as $h = (1 - \Sigma p_i^2) (n/n - 1)$, where p_i is the frequency of the ith allele and n is the number of ETs (20). Genetic distance between ETs was calculated as the proportion of fixed loci at which dissimilar alleles occurred, and the unweighted pair-group method of arithmetic averages clustering fusion strategy was used to create a phenogram (17). For the purpose of analysis, results for the human spirochetes were pooled with those previously obtained for 188 porcine spirochetes (16), and the resulting phenogram is presented as Fig. 1.

DNA hybridization. DNA was extracted from 14 selected isolates (see Table 3) by using techniques described for porcine intestinal spirochetes (3). These spirochetes include four strains from Italian adults (26), three from aboriginal children (15), two porcine isolates from group C in Fig. 1 (M1 and P43/6/78), the type strain of *S. innocens* (B256), the type strain of *S. hyodysenteriae* (B78), and three other well-characterized strains of *S. hyodysenteriae* (P18A, B204, and B234) (16). Briefly, cells pelleted from broth were lysed with lysozyme, proteinase K, and sodium dodecyl sulfate (SDS), and the DNA was extracted with phenol-chloroform-isoamyl alcohol. After dialysis, the solution was digested with RNase A, and the DNA was precipitated with ice-cold absolute ethanol containing 3 M sodium acetate, spooled out on a glass rod, and redissolved in sterile distilled water. DNA concentrations were measured by using fluorometry.

DNA from human spirochetal strain HRM7 and from *S. hyodysenteriae* P18A was then sonicated at 20 W for 2 min with a model 1510 Labsonic sonicator (B. Braun, Melsungen, Germany), with a 3-mm-diameter probe, to give an average fragment length of 10^3 bp as determined by agarose gel electrophoresis with *Hind*III-digested phage λ DNA molecular size markers. A 0.5-μg sample of this sonicated DNA in sterile distilled water was then labeled by nick translation (23) by using a commercial nick translation kit (Amersham International, Amersham, England). The precursor mixture contained 1 μmol each of dGTP, dTTP, and dATP and 15 μCi of [α-^{32}P]dCTP (3,000 Ci/mmol, 10 mCi/ml) in a total volume of 50 μl. The reaction was started by the addition of 2.5 U of DNA polymerase I and 50 pg of DNase I. This mixture was incubated at 15°C for 1.5 h, and the reaction was stopped by 10 min of incubation at 100°C. Labeled DNA was separated from unincorporated nucleotides by using a Sephadex G-50 column. The specific activity of the labeled DNA solutions was in excess of 10^7 cpm/μg as assessed by scintillation counting.

The membrane hybridization technique that was then used was based on standard methods (22, 25). A 0.5-μg sample of unlabeled DNA from these two strains and from each of the six other human and porcine strains shown in Table 3 were first heated in a boiling water bath for 5 min to convert the DNA to its single-stranded form. The tubes were then cooled on ice for 3 min, and 1 volume of 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) was added before the samples were placed in triplicate onto Hybond-N+ nylon membranes (Amersham International) by using a Bio-dot microfiltration apparatus (Bio-Rad Laboratories, Richmond, Va.). Each membrane was then placed DNA side up on three
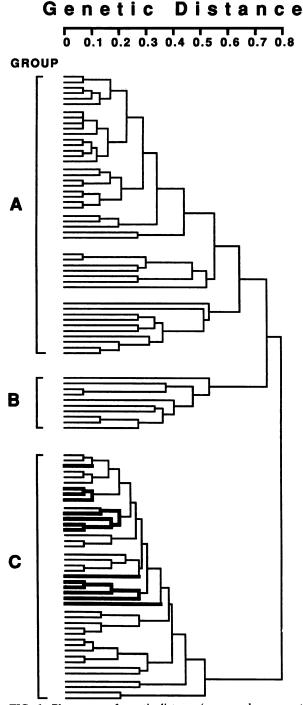
Genetic Distance

FIG. 1. Phenogram of genetic distance (expressed as percentage of fixed allelic differences) among ETs of 188 porcine and 35 human intestinal spirochetes, clustered by the unweighted pair-group method of arithmetic averages strategy. Group C (ETs 57 to 99) contains all of the human isolates in the 14 ETs outlined in bold. All strains of *S. hyodysenteriae* and *S. innocens* are located in group A.

sheets of Whatman no. 2 filter paper soaked in denaturing solution (1.5 M NaCl, 0.5 M NaOH) for 5 min. The membrane was then transferred to three sheets of filter paper soaked in neutralization solution (1.5 M NaCl, 0.5 M Tris-HCl [pH 7.2], 0.001 M EDTA) for 1 min. The DNA was then

fixed to the membrane by placing it on the filter paper soaked in 0.4 M NaOH for 20 min.

Before hybridization, the membranes were placed in a polyethylene bag and prehybridized at 68°C for 1 h with 15 ml of hybridization buffer consisting of 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7]), 1% SDS, 0.5% low-fat skim milk powder, and 0.5 mg of denatured herring sperm DNA per ml.

The radioactively labeled probe DNA was denatured by boiling for 5 min, cooled on ice, and then added to the hybridization buffer surrounding the membranes. The membranes were then incubated for 16 h at 68°C in a shaking water bath. After hybridization, the membranes were washed twice for 10 min at room temperature in 2× SSPE–0.1% SDS and then once for 15 min at 68°C in 1× SSPE–0.1% SDS. Autoradiographs were then made from the membranes (4 h of exposure at −70°C).

Individual dots containing the hybridized DNA were then cut from the membrane and placed in 3 ml of scintillation fluid in scintillation vials, and their radioactivity was measured in a liquid scintillation counter (model LS 3801; Beckman Instruments, Palo Alto, Calif.). The reading for the dot for each strain was then calculated as a percentage of the mean of five readings for DNA from the strain from which the particular probe was prepared. The mean of these values for each of three replicates for each strain was then recorded as the percentage of homology for that strain relative to the strain from which the probe was made.

RESULTS

Morphological and biochemical features. The phenotypic properties of the human intestinal spirochetes are presented in Table 1. All of the spirochetes were weakly beta-hemolytic. Sixteen (46%) produced indole, of which 10 belonged to ET 69. Of the 20 isolates tested in API-ZYM, 13 (65%) had α-galactosidase but not α-glucosidase activity, 1 had neither enzyme, and 6 had α-glucosidase but not α-galactosidase activity. All of the latter isolates also belonged to ET 69. All of the isolates that were examined under the electron microscope had four, five, or occasionally six axial filaments inserted subterminally in a single row. Some of these isolates are also quoted in the literature as having four to six axial filaments (5). In previous studies, we have shown that porcine spirochetes that belong to group C of Fig. 1 have a similar number of axial filaments inserted in the same way as those of the human isolates, whereas spirochetes from groups A and B have between 7 and 14 subterminal axial filaments inserted in two rows (16). The spirochetes in group C, from both swine and humans, also have more pointed ends than the porcine spirochetes in groups A and B and are thinner (0.19 to 0.27 μm compared to 0.29 to 0.4 μm) (16).

MEE. The 35 human spirochetes were distributed into 14 ETs, all clustered in group C on the phenogram that was produced (Fig. 1). Altogether, the phenogram comprised 99 ETs, of which group C contained ETs 57 to 99. Fifty-six porcine spirochetes were clustered in the other 29 ETs of group C. Multilocus genotypes for group C are presented in Table 2, and although many isolates from humans and swine were clearly closely related, no ET contained isolates from both species. Each of the six Italian strains had a unique ET, but the 29 isolates from aboriginal children were all located in eight ETs (Table 1). One ET (no. 69) contained 10 isolates, whereas two others (no. 70 and 79) contained 6 and 5 isolates, respectively. The first four isolates in ET 70 were from a pair of siblings, sampled in consecutive years (15).

TABLE 1. ET and biochemical and morphological features of human intestinal spirochetes

ET	Strain designation	Indole[a]	α-Galacto-sidase[a]	α-Gluco-sidase[a]	No. of axial filaments
59	HRM5	0	1	0	4–6[b]
63	Mar	1	NT	NT	4–6
64	Kar	1	1	0	4–6
65	HRM7	0	1	0	4–6[b]
67	HRM2	0	1	0	4–6[b]
68	HRM1	0	1	0	4–5[b]
69	Que	1	0	1	4–5
	Dis	1	0	1	NT
	WesA	1	0	1	NT
	Mel	1	0	1	NT
	Kid	1	0	1	NT
	Vri	1	0	1	NT
	Lev	1	NT	NT	NT
	Tum	1	NT	NT	NT
	Sta	1	NT	NT	4–6
	Sha	1	NT	NT	4–6
70	MargA	0	1	0	5–6
	MargB	0	1	0	NT
	MatA	0	1	0	NT
	MatB	0	1	0	NT
	Mar	0	NT	NT	NT
	Cis	0	NT	NT	NT
78	HRM14	0	1	0	4–6[b]
79	WesB	0	1	0	4–5
	VirA	0	1	0	NT
	Joy	0	NT	NT	5–6
	Let	0	NT	NT	NT
	Ama	0	NT	NT	NT
80	Nao	1	0	0	4–6
81	JerA	1	NT	NT	4–5
82	Cha	0	NT	NT	4–6
	Rus	0	NT	NT	NT
	Cut	0	NT	NT	NT
83	HRM4	1	1	0	4–6[b]

[a] Biochemical reactivities: 1, positive; 0, negative. NT, not tested.
[b] Values for Italian strains are from reference 5.

Details of the porcine spirochetes in groups A and B have been given elsewhere (16). Briefly, group A contained 98 isolates of strongly beta-hemolytic *S. hyodysenteriae* in the first 29 ETs, seven indole-positive weakly beta-hemolytic spirochetes in the next seven ETs (*"Serpulina intermedius"*), and 10 isolates of *S. innocens* in the last 10 ETs in the group. Group B contained 17 weakly beta-hemolytic spirochetes that phenotypically resembled *S. innocens*.

DNA hybridization. The results of DNA hybridization are presented in Table 3 together with values obtained for certain of these strains in a previous study (2). The radiolabeled DNA from human strain HRM7 showed a high level of homology with the other human strains (range, 67.1 to 126.1%) and with the two porcine spirochetes from group C

TABLE 2. Alleles at 15 enzyme loci for group C spirochetes shown in Fig. 1

ET[a]	No. of isolates	No. of alleles at the enzyme locus[b]														
		ACP	ADH	AK	ALP	EST	FDP	GDA	GDH	GPI	HK	MPI	NP	PEP	PGM	SOD
57	2	6	3	4	1	4	4	5	2	5	1	2	4	3	2	2
58	1	6	3	4	1	4	4	5	2	5	2	2	4	3	2	2
59	1	6	3	4	1	4	4	6	2	5	1	2	4	3	2	2
60	7	6	3	4	1	4	2	5	2	5	1	2	4	2	2	2
61	1	6	3	4	1	4	4	5	2	5	1	2	4	2	2	2
62	3	6	3	4	1	4	4	5	2	5	1	4	4	2	2	2
63	1	6	3	4	1	4	3	5	2	4	1	2	4	3	2	2
64	2	6	3	4	1	5	3	5	2	4	1	2	4	3	2	2
65	1	6	3	4	1	4	3	5	2	5	1	2	4	3	2	2
66	1	6	3	2	1	4	3	4	2	5	1	2	4	2	2	2
67	1	6	3	1	1	4	3	5	2	5	1	2	4	2	2	2
68	1	6	3	2	1	4	4	4	2	5	1	2	4	3	2	2
69	10	6	3	2	1	4	3	6	2	4	1	2	4	3	2	2
70	6	6	3	2	1	4	3	6	2	5	1	2	4	3	2	2
71	2	6	3	4	1	4	3	4	2	5	1	2	4	4	2	1
72	1	6	3	4	1	3	4	4	2	5	1	2	4	4	2	2
73	2	6	3	4	1	4	4	4	2	5	1	2	4	4	2	2
74	2	6	3	1	1	4	3	3	2	5	1	2	4	3	2	1
75	1	6	3	4	1	4	4	3	2	5	1	2	4	3	3	1
76	5	6	3	4	1	4	3	4	2	5	1	2	4	3	3	2
77	3	6	3	4	1	4	3	4	2	5	1	2	4	3	3	1
78	1	6	3	4	1	5	4	6	2	5	1	2	4	3	3	2
79	5	6	3	4	1	4	3	6	1	5	1	2	4	3	2	2
80	1	6	3	4	1	4	3	6	1	5	1	2	4	2	2	2
81	1	5	3	4	1	4	3	6	1	4	1	2	4	3	2	2
82	3	6	3	2	1	4	3	4	1	5	1	4	4	3	2	2
83	1	6	3	2	1	5	4	5	1	5	1	2	4	2	2	2
84	1	6	3	4	1	5	3	5	2	5	1	2	4	2	1	1
85	3	6	3	4	1	5	3	5	2	5	1	2	4	4	3	1
86	1	6	3	4	1	4	3	5	2	5	2	4	4	2	1	1
87	1	6	3	4	1	4	3	6	2	5	2	2	4	2	1	1
88	1	6	3	4	1	4	3	4	2	5	2	2	4	2	1	1
89	1	6	3	4	1	4	4	4	2	5	1	2	4	2	3	2
90	1	7	3	4	1	4	4	4	2	5	1	2	4	2	3	2
91	1	7	3	4	1	4	4	4	2	5	1	6	4	2	3	2
92	2	7	3	4	1	4	4	4	2	5	1	6	4	2	4	1
93	4	7	3	4	1	4	4	4	2	5	1	2	4	2	4	1
94	1	6	3	4	1	5	4	4	2	5	1	7	4	5	4	2
95	1	7	3	4	1	4	4	4	2	5	1	1	5	5	4	2
96	1	5	3	4	1	4	3	5	2	5	1	3	4	2	3	2
97	2	5	4	4	1	6	3	3	2	5	1	6	4	2	3	2
98	2	3	3	2	1	6	4	2	2	5	1	7	4	3	3	2
99	1	7	3	2	1	6	3	2	2	5	3	7	4	3	3	2

[a] ETs that are underlined contain human spirochetes.

[b] Abbreviations: ACP, acid phosphatase; ADH, alcohol dehydrogenase; AK, adenylate kinase; ALP, alkaline phosphatase; EST, esterase; FDP, fructose-1,6-disphosphatase; GPI, glucose phosphate isomerase; GDA, guanine deaminase; GDH, glutamate dehydrogenase; HK, hexokinase; MPI, mannose-6-phosphate isomerase; NP, nucleoside phosphorylase; PEP, L-leucyl-glycylglycine peptidase; PGM, phosphoglucomutase; SOD, superoxide dismutase.

on the phenogram (83.5 and 128.9%) but gave low values with the type strain of *S. innocens* (strain B256, 18.6%), the type strain of *S. hyodysenteriae* (strain B78, 8.0%), and three other strains of *S. hyodysenteriae* (range, 11.3 to 18.4%). Conversely, radiolabeled DNA from *S. hyodysenteriae* P18A gave high values with the other three strains of *S. hyodysenteriae* (range, 88.3 to 104.1%) but low values with the other porcine spirochetes and with the human strains (range, 5.2 to 11.9%).

DISCUSSION

This study has demonstrated that the human intestinal spirochetes studied differ from *S. hyodysenteriae* and *S. innocens* both morphologically and genetically. *Serpulina* spp. have 7 to 14 axial filaments inserted subterminally in two rows at both ends, whereas the human spirochetes, in common with the porcine spirochetes in group C, all had 4, 5, or occasionally 6 filaments inserted in one row. Similar observations have been made by other workers (2, 5). The human spirochetes also tended to be more slender than porcine spirochetes in groups A and B (which contained the species *S. hyodysenteriae* and *S. innocens*), and their ends were more pointed. The biochemical reactivities of the human spirochetes varied, but it was of interest that all of the aboriginal isolates in ET 69 produced indole and had α-glucosidase but not α-galactosidase activity. These are features usually associated with the strongly beta-hemolytic *S. hyodysenteriae* (9), although they are also shared with the weakly beta-hemolytic spirochetes in ETs 30 to 36 of group A (16).

Results of MEE analysis clearly grouped spirochetes isolated from Australian aboriginal children and from Euro-

TABLE 3. DNA-DNA hybridization with the genomes of human and porcine intestinal spirochetes and comparison with published results

Source of unlabeled DNA	% Reassociation[a] with labeled DNA from:		
	HRM7	P18A	P18A[b]
Human spirochetes			
Italian			
HRM7 (ET 65)	100	8.6 ± 0.12	100.6 ± 6.3
HRM2 (ET 67)	99.9 ± 5.50	8.2 ± 0.01	90.5 ± 7.5
HRM4 (ET 83)	99.5 ± 1.09	11.9 ± 0.42	104.8 ± 9.7
HRM14 (ET 78)	95.4 ± 0.83	7.6 ± 0.30	55.6 ± 15.8
Australian			
WesA (ET 69)	67.1 ± 1.59	5.2 ± 0.20	ND
WesB (ET 79)	100.2 ± 6.30	9.7 ± 0.64	ND
Roch (ET 82)	126.1 ± 9.90	11.2 ± 0.28	ND
Porcine spirochetes			
Group C			
M1 (ET 66)	83.5 ± 7.60	17.8 ± 2.35	93.3 ± 19.9
P43/6/78 (ET 61)	128.9 ± 5.30	19.4 ± 2.04	ND
S. innocens B256 (ET 36)	18.6 ± 0.13	14.5 ± 0.90	ND
S. hyodysenteriae			
P18A (ET 15)	18.4 ± 1.20	100	100
B78 (ET 5)	8.0 ± 2.00	88.3 ± 7.10	ND
B204 (ET 18)	11.3 ± 1.20	104.1 ± 1.00	ND
B234 (ET 1)	11.7 ± 1.20	101.4 ± 6.80	ND

[a] Mean ± standard deviation of three separate reactions relative to the labeled reference strain.
[b] Values are from reference 2. ND, not determined.

pean adults closely together and demonstrated their genetic similarity to certain porcine spirochetes. These spirochetes in group C of the phenogram were genetically distinct from the *Serpulina* spp. which were located in group A (16).

Since these results were at odds with two previous studies where DNA from *S. hyodysenteriae* P18A gave high homology values with some of the same Italian human strains used in the present study (2, 6) (Table 3), we elected to check our MEE groupings by using DNA hybridization. The filter hybridization technique used by us was different from that employed by the previous workers, but the stringency of hybridization was the same (annealing temperature, T_m − 25°C). Results from this work clearly demonstrated that the human strains were closely related to porcine spirochetes of group C and not to *S. hyodysenteriae* as had been claimed (2, 6). Closer examination of these previous studies revealed two main weaknesses. In both previous studies, DNA from only one strain of so-called *S. hyodysenteriae* was used, and, in the larger study (2), porcine spirochete M1 was used as an example of *S. innocens*. Unfortunately, M1 is not *S. innocens* but has four or five axial filaments and belongs to group C on the phenogram (16). We suspect that the DNA from the so-called strain P18A that was used in these previous studies (at the same laboratory) was in fact not from *S. hyodysenteriae* but originated from a group C spirochete. Since DNAs from other genuine *S. hyodysenteriae* strains were not used as controls, this error went undetected and the wrong conclusions were drawn. This interpretation also explains the unexpectedly high homology values (93.3%) between the so-called *S. innocens* M1 and the so-called *S. hyodysenteriae* P18A found in that work (2). This would occur if both isolates actually belonged to the same species in group C.

The current study demonstrates that the human intestinal spirochetes closely resemble porcine spirochetes of group C. These porcine spirochetes include P43/6/78, in ET 61, the original isolate used to produce porcine intestinal spirochetosis (29), as well as numerous Australian isolates from pigs with similar conditions (16). While the clinical significance of intestinal spirochetosis in humans has been questioned (14, 21), many workers believe that the spirochetes are the cause of a variety of gastrointestinal disturbances, particularly chronic diarrhea and rectal bleeding (4, 7, 11, 26). The human spirochetes used in this study all came from individuals with diarrhea or intestinal disturbances (15, 26), and these fall within the general description of intestinal spirochetosis.

There are a number of similarities between intestinal spirochetosis in swine and that in humans. Both are associated with protracted diarrhea, with spirochetes attached end-on to the colonic epithelium (10, 14). Our demonstration that spirochetes recovered from the same condition in both species are genetically related now adds weight to the belief that these bacteria have a pathogenic role in humans. This is further supported by the finding of only a small number of spirochetal clones colonizing large numbers of children in a remote and extended aboriginal community (15). For example, 10 children were colonized by bacteria of ET 69, and six children were colonized by spirochetes of ET 70. These ETs only differed by a single allele at one enzyme locus. Furthermore, two siblings were found to be colonized by spirochetes of ET 70 when sampled in consecutive years, suggesting long-term colonization. All of these children had severe to moderate diarrhea at the time of fecal sampling (15).

In view of the widespread colonization by intestinal spirochetes in people from many parts of the world where chronic diarrhea is rife (1, 15, 18, 19), further study of the bacteria is warranted. The availability of a similar infection in swine may represent a useful experimental model of the condition.

ACKNOWLEDGMENTS

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