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Restriction Fragment Length Polymorphism of the Periplasmic Flagellar *flaA1* Gene of *Serpulina* Species‡

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Forty-one reference and field isolates of intestinal spirochetes representing *Serpulina hyodysenteriae*, *Serpulina innocens*, *Serpulina pilosicoli*, *Brachyspira aalborgi*, and nonclassified weakly beta-hemolytic intestinal spirochetes were compared by restriction fragment length polymorphism (RFLP) of the periplasmic flagellar (PF) *flaA1* gene. Six genetically distinct groups (I through VI), each with a unique RFLP fingerprint pattern, were identified by Southern blotting analysis of *Eco*RV chromosomal DNA digests with a PCR-amplified digoxigenin-labeled 1-kb fragment of the *S. hyodysenteriae* isolate B78 PF *flaA1* gene. The RFLP fingerprint patterns corresponded to known DNA homology differences between *Serpulina* species and to provisionally designated species described previously by using phenotypic and genotypic classification schemes. RFLP fingerprinting of the PF *flaA1* gene provides a relatively simple genotypic method for identification of intestinal spirochetes without the use of radioisotopes.

Three species of intestinal spirochetes have been taxonomically classified within the genus *Serpulina*. *Serpulina hyodysenteriae*, the etiologic agent of swine dysentery (7, 11, 19, 22, 40) is a strongly beta-hemolytic spirochete associated with a diarrheal disease of growing pigs that is of worldwide economic importance (34). The spirochete colonizes the cecum and colon; the colonization results in severe mucohemorrhagic typhlocolitis which can lead to dehydration and death if the pigs are left untreated. Other intestinal spirochetes are morphologically similar to *S. hyodysenteriae*, but they produce a weak beta-hemolysis when cultured anaerobically on agar medium containing blood (7). Because weakly beta-hemolytic intestinal spirochetes (WBHIS) were originally isolated from the colons of healthy swine, they were given the name *Serpulina innocens* (22). Recently, certain WBHIS have been associated with a diarrheal disease of humans and animals (7–9, 20, 24, 28, 31, 33, 37, 42, 44) and have been classified as *Serpulina pilosicoli* (44).

S. hyodysenteriae has been classified into serotypes (1 through 9) or serogroups (A through I) based on the reactivities of cell wall lipooligosaccharide antigens with hyperimmune sera (3, 14–18, 29, 32). Other proposed classifications of intestinal spirochetes have been based on multilocus enzyme electrophoresis (MEE), restriction endonuclease analysis, and restriction fragment length polymorphism (RFLP) of 16S rRNA, periplasmic flagellar (PF), and hemolysin genes (5, 6, 21, 26–28, 31, 35, 36, 38, 41–43). Each method has resulted in a classification scheme that confirms the diversity among intestinal spirochetes. Collectively these observations suggest the existence of new species in addition to taxonomically accepted *S. hyodysenteriae*, *S. innocens*, and *S. pilosicoli* (26, 28). Some studies have combined more than one method; Stanton and coworkers used MEE and 16S rRNA sequence comparisons (41), while Koopman and coworkers (24) used RFLP of hemolysin, PF, and 16S rRNA genes. On the basis of 16S rRNA sequence comparisons and biochemical analysis, three phylogenetic clusters containing four phenotypes have been identified among porcine intestinal spirochetes (12, 36). One cluster consisted of *S. hyodysenteriae* as phenotype I and WBHIS variants that were biochemically similar to *S. hyodysenteriae* as phenotype II. A second cluster, represented by phenotype III, was the most variable, with three subgroups, designated IIIa, IIIb, and IIIc, of *S. innocens* isolates. The third cluster consisted of phenotype IV and included WBHIS similar to isolate P43/6/78, the reference isolate for *S. pilosicoli* (44).

The most comprehensive classification scheme of intestinal spirochetes has used MEE to divide intestinal spirochetes into seven groups (I through VII), all of which have similar 16S rRNA sequences (26, 28, 31, 41). In this proposed classification *S. hyodysenteriae* forms group I, previously designated group Aa (26, 27, 31), *S. innocens* forms group III, previously designated group Ab (26), and *S. pilosicoli* forms group VI, previously designated group C (26). Other nonclassified WBHIS, provisionally designated "*Serpulina intermedius*" and "*Serpulina murdochii*," formed group II or Ab and group V or B, respectively (26, 28). A single isolate of *Brachyspira aalborgi*, which is associated with colonic spirochetal infections of humans and rhesus macaques (10, 20), forms group VII.

In this study we compared 41 reference and field isolates of intestinal spirochetes representing *S. hyodysenteriae*, *S. innocens*, *S. pilosicoli*, *B. aalborgi*, and nonclassified WBHIS by using RFLP of the PF *flaA1* gene. Fingerprint patterns were identified by Southern blotting analysis of *Eco*RV chromosomal DNA digests with a PCR-amplified digoxigenin (DIG)-labeled 1-kb fragment of the *S. hyodysenteriae* isolate B78 PF *flaA1* gene. The RFLP fingerprint patterns corresponded to *Serpulina* species identified by previously described phenotypic and genotypic methods. RFLP fingerprinting of the PF *flaA1* gene provided a relatively simple genotypic method for the identification of intestinal spirochetes without the use of radioisotopes.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. The sources, origins, and current classifications of reference and field isolates of intestinal spirochetes used in this study are described in Table 1. The spirochetes were propagated in prereduced anaerobically sterilized Trypticase soy broth as previously described (25). Cultures were grown to late log phase (approximately 10^8 cells per ml) in 5-ml volumes by using Hungate tubes and were stirred constantly at 37°C under an atmosphere of 10% hydrogen, 10% carbon dioxide, and 80% nitrogen.

Isolation of chromosomal DNA. Spirochetes were harvested (15,000 × *g*; 2 min), resuspended in TE buffer (10 mM Tris, 1 mM EDTA [pH 8.0]), and lysed by the addition of 0.6 mg of proteinase K (37°C, 1 h; Life Technologies, Gaithersburg, Md.). Polysaccharides and remaining proteins were removed by mixing the solution with 10% hexadecyltrimethyl ammonium bromide (Sigma, St. Louis, Mo.) in 0.7 M NaCl (65°C, 10 min), followed by extraction with chloroform-isoamyl alcohol and phenol-chloroform (2). The DNA was precipitated with isopropyl alcohol, washed with 70% ethanol, and resuspended in TE buffer. Contaminating RNA was removed by the addition of 1.25 μg of RNaseA–5 U of $RNaseT_1$ (37°C, 1 h; Sigma), followed by ethanol precipitation. The resulting chromosomal DNA was resuspended in TE buffer, and the concentration was determined by fluorometry against a *Clostridium perfringens* DNA standard in a (Dyna Quant; Hoeffer Pharmacia, San Francisco, Calif.).

Restriction endonuclease digestion and Southern blotting. A total of 2 μg of chromosomal DNA from each spirochete was digested with 10 U of restriction enzyme *Eco*RV (37°C, 1 h; Life Technologies) and separated on 0.8% agarose gels with 16 mM Tris base–8 mM sodium acetate–1 mM EDTA (TAE running buffer) (2). The gels were run at 90 V for 2 h at room temperature (RT), stained with ethidium bromide in TAE running buffer for 15 min, and photographed under UV light. The digested DNA fragments were transferred by capillary diffusion to nylon membranes (Hybond-N; Amersham, Arlington, Ill.) and fixed by irradiation with UV light for 10 min (2, 39).

Preparation of DIG-labeled PF *flaA1* probe. The PF *flaA1* gene of *S. hyodysenteriae* B78 was amplified with an oligonucleotide primer pair (positive sense, 5′-GGAATTCCATGGAAAAGTTATTCGTAGTATTAACTTCC-3′ [*flaIA*] and negative sense 5′-CGAATTCTAGATTATTGAGCTTGTTCTTGAGCAGC-3′ [*flaIB*]) designed and synthesized (Integrated DNA Technologies, Coralville, Iowa) on the basis of the published DNA sequence for the PF *flaA1* gene of *S. hyodysenteriae* isolate C5 (23). Purified chromosomal DNA (100 ng) was reacted with 200 pmol of each primer in a total volume of 50 μl containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 4 mM $MgCl_2$ with 200 μM concentrations of dATP, dCTP, dGTP, and dTTP (Promega, Madison, Wis.), 0.02 mM DIG-11-dUTP (Boehringer Mannheim, Indianapolis, Ind.), and 2.5 U of *Taq* polymerase (Life Technologies); the reaction consisted of 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min. A control reaction without the DIG-11-dUTP was carried out to evaluate the incorporation of the DIG label. The PCR products were visualized by electrophoresis on 0.8% agarose gels, with ethidium bromide staining as described above. The resulting DIG-labeled PF *flaA1* probe was stabilized with 0.02 M EDTA and stored at 4°C until needed.

DNA hybridization and immunodetection. Fragments of digested chromosomal DNA from each spirochete immobilized on filters were probed and visualized by following the manufacturer's recommended procedure (4). Briefly, membranes were prehybridized in 5× SSC (1× SSC is 0.15 M NaCl plus 15 mM sodium citrate, pH 7.0)–0.1% *N*-lauroylsarcosine (Sigma)–0.02% sodium dodecyl sulfate (SDS; J. T. Baker, Phillipsburg, N.J.)–1% blocking reagent (Boehringer Mannheim) for 6 h at 45°C. After being denatured (100°C, 10 min), the DIG-labeled probe (2 μg) was held on wet ice until being mixed with the prehybridization buffer. The DIG-labeled probe was hybridized to the immobilized DNA fragments for 18 h at 45°C. The membranes were washed twice with 2× SSC–0.1% SDS for 5 min each at RT followed by two washes with 0.5× SSC–0.1% SDS for 15 min each at 45°C. The membranes were equilibrated in wash solution (100 mM maleic acid [Sigma], 150 mM NaCl with 0.3% Tween 20 [Sigma]) for 2 min at RT and blocked with 1% blocking reagent in 100 mM maleic acid–150 mM NaCl (blocking buffer) for 30 min at RT. Bound DIG-labeled probe was incubated with sheep anti-DIG Fab fragments conjugated to alkaline phosphatase (Boehringer Mannheim) in blocking buffer for 30 min at RT. After being washed twice for 15 min at RT, the bound DIG-labeled probe was visualized by being stained with 5-bromo-4-chloro-3-indolylphosphate–nitroblue tetrazolium substrate (Kirkegaard and Perry, Gaithersburg, Md.). Approximate band sizes were determined relative to the migration of a 1-kb DNA ladder marker (Life Technologies).

RESULTS

RFLP fingerprint pattern of *S. hyodysenteriae* strains. Hybridization of the DIG-labeled *S. hyodysenteriae* isolate B78 PF *flaA1* probe with restriction-digested chromosomal DNA from reference and field isolates of *S. hyodysenteriae* produced bands of approximately 5.8 and 3.5 kb (Fig. 1). This RFLP fingerprint pattern was designated pattern I. Strain WA-6, the reference isolate for serogroup E, consistently yielded an additional band of approximately 8 kb.

RFLP fingerprint pattern of *S. innocens* strains. Hybridization of the DIG-labeled *S. hyodysenteriae* isolate B78 PF *flaA1* probe with restriction-digested chromosomal DNA from reference isolates of *S. innocens* produced bands of approximately 5.8 and 2.9 kb (Fig. 2), a pattern designated pattern III. The lower band of *S. innocens* C301 corresponded to a molecular weight slightly lower than those for all other strains of the same species.

RFLP fingerprint pattern of *S. pilosicoli* strains. Hybridization of the DIG-labeled *S. hyodysenteriae* isolate B78 PF *flaA1* probe with restriction-digested chromosomal DNA from *S. pilosicoli* isolates produced bands of approximately 3.4 and 2.6 kb (Fig. 3–5). With the exception of macaque *S. pilosicoli* isolate MMU26717, which consistently yielded bands of approximately 9.0 and 2.6 kb (Fig. 5), there were no differences in the hybridization patterns based on the host from which the strain had been isolated. This RFLP fingerprint pattern was designated pattern V.

RFLP fingerprint pattern of *B. aalborgi*. Hybridization of the DIG-labeled *S. hyodysenteriae* isolate B78 PF *flaA1* probe with restriction-digested chromosomal DNA from *B. aalborgi* produced a single band, and this pattern was designated pattern VI (Fig. 4).

RFLP fingerprint patterns of nonclassified WBHIS. Hybridization of the DIG-labeled *S. hyodysenteriae* isolate B78 PF *flaA1* probe with restriction-digested chromosomal DNA from WBHIS isolates provisionally designated "*S. intermedius*" produced bands of approximately 2.7 and 2.0 kb (Fig. 6), whereas WBHIS isolates provisionally designated "*S. murdochii*" produced two distinct banding patterns. Isolate 155-20 had bands of approximately 6.1 and 4.5 kb, and isolate 56-150 had bands of approximately 5.0 and 2.7 kb (Fig. 6). These RFLP fingerprint patterns were designated patterns II, IVa, and IVb, respectively.

DISCUSSION

Koopman and coworkers (24) and Li and coworkers (30) reported that PF protein FlaA1 has the greatest variability among the five PF proteins of intestinal spirochetes. We have shown that the PF FlaA1 protein is antigenically similar among porcine *Serpulina* species, as demonstrated by the immunoblot reactivity of an FlaA1-specific rabbit antiserum to the 44-kDa PF protein of intestinal spirochetes (13, 30). However, Koopman and coworkers (24) suggested that there was polymorphism of the PF *flaA1* gene among porcine *S. hyodysenteriae* isolates B204 and C5, *S. innocens* isolate B256, human *B. aalborgi*, and 24 nonclassified WBHIS isolated from humans ($n = 10$), dogs ($n = 5$), rats ($n = 4$), mice ($n = 4$), and a chicken. In that study, the chromosomal DNA of each spirochete digested with *Eco*RV was probed with the PF *flaA1* gene of *S. hyodysenteriae* isolate C5. Using the PF *flaA1* gene as a probe and chromosomal DNA digested with *Taq*I, ter Huurne and coworkers (43) identified seven RFLP fingerprint patterns among 10 nonclassified porcine WBHIS isolates, in addition to *S. innocens*. In the same study, 51 reference and field isolates of *S. hyodysenteriae* were divided into two groups on the basis of RFLP fingerprint patterns. In the present study, we used the PF *flaA1* gene from reference *S. hyodysenteriae* isolate B78 to evaluate DNA polymorphism within the genes encoding the PF FlaA1 proteins of 41 well-characterized intestinal spirochetes representing reference and field isolates of *Serpulina* species and *B. aalborgi*. Our results confirmed those of Koopman and coworkers (24) and Li and coworkers (30), but further

TABLE 1. Sources origins, and current and proposed phenotypic and genotypic classifications of intestinal spirochetes investigated in this study

Species and strain	Origin (animal/country[i])	Phenotype/ MEE type[a]	Serogroup/ serotype[b]	RFLP *flaA1* pattern	Source/reference(s)[c]
S. hyodysenteriae[d]					
B78 (ATCC 27164^T)	Pig/U.S.	I/Aa-I	A/1	I	1/3, 18, 21, 40
B204	Pig/U.S.	I/Aa-I	B/2	I	1/3, 40
B169	Pig/Canada	I/Aa	C/3	I	1/3
A1	Pig/U.K.	I/Aa	D/4	I	1/3
WA-6	Pig/Australia	I/Aa	E/ND[e]	I	2/14, 15, 27
Q16	Pig/Australia	I/Aa	G/ND	I	2/14–16, 27
VIC2	Pig/Australia	I/Aa	H/ND	I	2/14–16, 27
NSW-1	Pig/Australia	I/Aa	I/ND	I	2/14–16, 27
B8044	Pig/U.S.	I/Aa	B/5	I	3/18, 32
B6933	Pig/U.S.	I/Aa	A/6	I	3/18, 32
Ack 300/8	Pig/The Netherlands	I/Aa	B/7	I	3/18, 32
FM88-90 (ATCC 49887)	Pig/Canada	I/Aa	J/8	I	4/18, 29
FMV89-3233 (ATCC49886)	Pig/Canada	I/Aa	K/9	I	4/18, 29
"*S. intermedius*"					
889	Pig/Australia	II/Ab	NA[f]	II	2/26
UNL-2	Pig/U.S.	II/ND	NA	II	5/33, 37
AN26:93	Pig/Sweden	II/ND	NA	II	6/36
S. innocens					
C301	Pig/Sweden	IIIa/ND	NA	III	6/36
C378	Pig/Sweden	IIIa/ND	NA	III	6/36
C336	Pig/Sweden	IIIb/ND	NA	III	6/36
B256 (ATCC 29786^T)	Pig/U.S.	IIIc/Ac-III	NA	III	7/22
4/71	Pig/U.K.	IIIb/Ac	NA	III	8/22, 26, 37
"*S. murdochii*"					
155-20	Pig/Australia	III/B-V	NA	IVa	2/26, 41
56-150	Pig/Canada	III/B	NA	IVb	9/26
S. pilosicoli[g]					
P43/6/78 (ATCC 49776^T)	Pig/U.K.	IV/C-VI	NA	V	8/26, 36, 41, 44
UNL-3	Pig/U.S.	IV/C-VI[h]	NA	V	5/33, 37
UNL-5	Pig/U.S.	IV/C-VI[h]	NA	V	5/33, 37
UNL-8	Pig/U.S.	IV/ND	NA	V	5/33
D9201243A	Pig/U.S.	IV/ND	NA	V	10/8, 33
T9300098	Pig/U.S.	IV/ND	NA	V	10/8, 33
T9301604B	Pig/U.S.	IV/ND	NA	V	10/8, 33
B359	Pig/U.S.	IV/C-VI[h]	NA	V	5/33, 37
B1555a	Pig/U.S.	IV/C-VI[h]	NA	V	5/33, 37
Meyers K9-12	Dog/U.S.	IV/C-VI[h]	NA	V	11/9
16242-94	Dog/U.S.	IV/C-VI[h]	NA	V	5/9
Dog 17	Dog/Papua New Guinea	IV/C-VI[h]	NA	V	2/28
SP16	Human/U.S.	IV/C-VI	NA	V	12/9, 28, 37
HRM5B	Human/Italy	IV/C-VI	NA	V	2/5, 28
MMU26717	Primate/U.S.	IV/ND	NA	V	5/10
MMU26986	Primate/U.S.	IV/ND	NA	V	5/10
MMU27669	Primate/U.S.	IV/ND	NA	V	5/10
B. aalborgi					
513A (ATCC 43994^T)	Human/Denmark	NA/VII	NA	VI	2/20, 41

[a] Phenotype according to Fellström and coworkers (12) and MEE type according to Lee and coworkers (26, 27) and Stanton and coworkers (41).

[b] *S. hyodysenteriae* serogrouping according to Hampson et al. (14–16, 18) and serotyping according to Baum and Joens (3), Mapother and Joens (32), and Li and coworkers (29).

[c] 1, J. M. Kinyon, College of Veterinary Medicine, Iowa State University, Ames; 2, D. J. Hampson, School of Veterinary Studies, Murdoch University, Western Australia, Australia; 3, L. A. Joens, Department of Veterinary Science, University of Arizona, Tucson; 4, M. Jacques, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada; 5, G. E. Duhamel, Department of Veterinary and Biomedical Sciences, University of Nebraska—Lincoln; 6, C. Fellström, Department of Medicine and Surgery, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden; 7, American Type Culture Collection, Rockville, Md.; 8, T. B. Stanton, National Animal Disease Center, Ames, Iowa; 9, S. Messier, Faculté de Médecine Vétérinaire, Université de Montréal; 10, R. L. Walker, California Veterinary Diagnostic Laboratory System, University of California—Davis; 11, M. J. Wannemuehler, College of Veterinary Medicine, Iowa State University; 12, R. M. Smibert, Virginia Polytechnic Institute, Blacksburg.

[d] All strains are positive by PCR amplification of an *S. hyodysenteriae*-specific chromosomal gene (11).

[e] ND, not determined.

[f] NA, not applicable.

[g] All strains are positive by PCR amplification of an *S. pilosicoli*-specific 16S rRNA gene (33).

[h] See reference 15a.

[i] U.S., United States; U.K., United Kingdom.

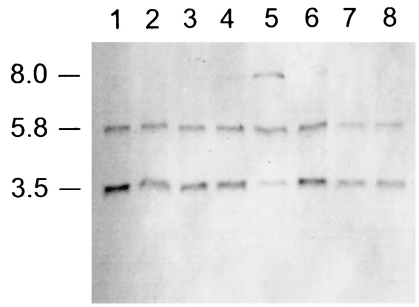

FIG. 1. Hybridization of the DIG-labeled *S. hyodysenteriae* isolate B78 PF *flaA1* probe with *Eco*RV-digested chromosomal DNA from reference isolates of porcine *S. hyodysenteriae*. Lane 1, isolate B78; lane 2, isolate B204; lane 3, isolate B169; lane 4, isolate A1; lane 5, isolate WA-6; lane 6, isolate Q16; lane 7, isolate VIC2; lane 8, isolate NSW-1. The apparent molecular sizes are indicated at the left in kilobase pairs.

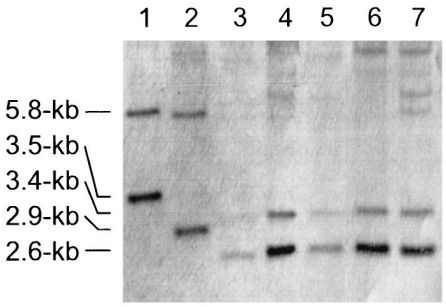

FIG. 3. Hybridization of the DIG-labeled *S. hyodysenteriae* isolate B78 PF *flaA1* probe with *Eco*RV-digested chromosomal DNA from reference and field isolates of porcine *S. pilosicoli*. Lane 1, *S. hyodysenteriae* isolate B78; lane 2, *S. innocens* isolate B256; lane 3, *S. pilosicoli* isolate P43/6/78; lane 4, *S. pilosicoli* isolate UNL-8; lane 5, *S. pilosicoli* isolate T9301604B; lane 6, *S. pilosicoli* isolate B359; lane 7, *S. pilosicoli* isolate B1555a. The apparent molecular sizes are indicated at the left in kilobase pairs.

studies with isolates representing all known species and provisionally designated species of intestinal spirochetes indicated a high degree of correlation with taxonomically accepted species designation and classification schemes using phenotypic methods (12, 26, 36), MEE (26, 28, 41), and sequence of 16S rRNA (12, 33, 36, 41).

Preliminary experiments indicated that PCR amplification of the PF *flaA1* gene generated products of approximately 1 kb with chromosomal DNA from all *S. hyodysenteriae* and *S. innocens* isolates, but not with DNA from *S. pilosicoli* (data not shown), confirming the heterogeneity of the PF *flaA1* gene sequence among *Serpulina* species. Under moderate-stringency conditions, the PF *flaA1* gene probe hybridized with all intestinal spirochetes evaluated including *B. aalborgi*, thus providing evidence of sequence homology. However, polymorphism of the PF *flaA1* gene among intestinal spirochetes was suggested on the basis of differences in RFLP fingerprint patterns.

Fingerprint pattern I applied to all *S. hyodysenteriae* reference isolates, with one variation observed with *S. hyodysenteriae* isolate WA-6, which consistently produced an additional higher-molecular-weight band. This may be attributable to incomplete restriction endonuclease digestion of WA-6 chromosomal DNA, perhaps because of site-specific methylation in this isolate.

Intestinal spirochetes provisionally designated "*S. intermedius*" had an RFLP fingerprint pattern distinctly different from that of other intestinal spirochetes; this pattern was designated RFLP pattern II. This finding supports previous reports indicating a distinct grouping of these spirochetes as group Ab or group II (26, 41) and phenotype II (12, 36). Pattern III included all *S. innocens* isolates examined, with a minor variation with *S. innocens* isolate C301. However, the PF *flaA1* RFLP fingerprint pattern of *S. innocens* isolates was not consistent with a previously proposed phylogenetic separation of *S. innocens* based on the 16S rRNA sequence (36). Although WBHIS isolates 155-20 and 56-150 belong to MEE genotype group B or group V and have been provisionally designated "*S. murdochii*" (26, 41), the RFLP fingerprint pattern of the PF *flaA1* gene of isolate 56-150 was similar to that of *S. innocens* isolate C301. This is in agreement with the proposal of Pettersson and coworkers (36), suggesting that spirochetes classified phenotype IIIa may be a subspecies of *S. innocens*. Collectively, these observations suggested that the provisional taxonomic group "*S. murdochii*" defined by MEE (26, 41) might include a subgroup of spirochetes that were found to be closely related to *S. innocens* when examined by 16S rRNA sequencing or RFLP of the PF *flaA1* gene. Further comparative analysis of additional WBHIS with RFLP fingerprint patterns III and IV using MEE

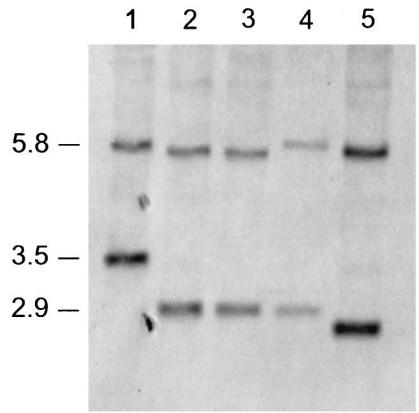

FIG. 2. Hybridization of the DIG-labeled *S. hyodysenteriae* isolate B78 PF *flaA1* probe with *Eco*RV-digested chromosomal DNA from reference and field isolates of porcine *S. innocens*. Lane 1, *S. hyodysenteriae* isolate B78; lane 2, *S. innocens* isolate B256; lane 3, *S. innocens* isolate 4/71; lane 4, *S. innocens* isolate C336; lane 5, *S. innocens* isolate C301. The apparent molecular sizes are indicated at the left in kilobase pairs.

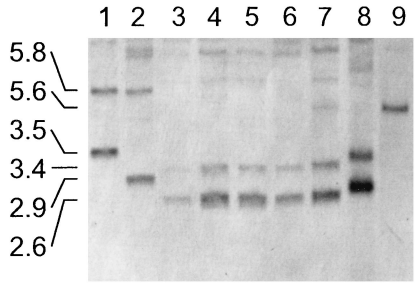

FIG. 4. Hybridization of the DIG-labeled *S. hyodysenteriae* isolate B78 PF *flaA1* probe with *Eco*RV-digested chromosomal DNA from canine and human *S. pilosicoli* isolates and *B. aalborgi*. Lane 1, *S. hyodysenteriae* isolate B78; lane 2, *S. innocens* isolate B256; lane 3, porcine *S. pilosicoli* isolate P43/6/78; lane 4, canine *S. pilosicoli* isolate Meyers K9-12; lane 5, canine *S. pilosicoli* isolate 16242-94; lane 6, canine *S. pilosicoli* isolate Dog 17; lane 7, human *S. pilosicoli* isolate SP16; lane 8, human *S. pilosicoli* isolate HRM5B; lane 9, human *B. aalborgi*. The apparent molecular sizes are indicated at the left in kilobase pairs.

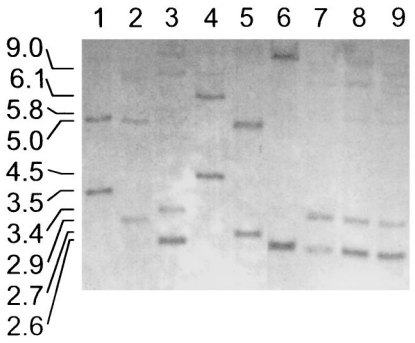

FIG. 5. Hybridization of the DIG-labeled *S. hyodysenteriae* isolate B78 PF *flaA1* probe with *Eco*RV-digested chromosomal DNA from reference and field isolates of nonclassified porcine intestinal spirochetes and porcine and rhesus macaque *S. pilosicoli* isolates. Lane 1, *S. hyodysenteriae* isolate B78; lane 2, *S. innocens* isolate B256; lane 3, *S. pilosicoli* isolate P43/6/78; lane 4, "*S. murdochii*" isolate 155-20; lane 5, "*S. murdochii*" isolate 56-150; lane 6, rhesus macaque *S. pilosicoli* isolate MMU26717; lane 7, porcine *S. pilosicoli* isolate UNL-5; lane 8, porcine *S. pilosicoli* isolate T9300098; lane 9, porcine *S. pilosicoli* isolate UNL-3. The apparent molecular sizes are indicated at the left in kilobase pairs.

or 16S rRNA sequencing will be necessary in order to clarify this finding.

With the exception of rhesus macaque isolate MMU26717, *S. pilosicoli* isolates obtained from a wide range of hosts had similar RFLP fingerprint patterns. The higher-molecular-weight band of isolate MMU26717 may be attributable to incomplete digestion of chromosomal DNA, perhaps because of methylation at this site. These results confirmed previous reports indicating that *S. pilosicoli* belongs to a distinct MEE genotype (26, 31, 36, 41).

Based on the sequence of the PF *flaA1* gene of *S. hyodysenteriae* isolate C5 (23), *Eco*RV is predicted to cut once within the gene at nucleotide position 864. The RFLP fingerprint patterns of reference and field isolates of *Serpulina* species and nonclassified WBHIS were consistent with this observation; two hybridization bands were present for each spirochete. Conversely, only one hybridization band was present for *B. aalborgi* (Fig. 4), indicating that this isolate lacks the *Eco*RV restriction site within the PF *flaA1* gene. This finding confirms that the *B. aalborgi* PF *flaA1* gene is genetically distinct from those of *Serpulina* species (28, 36, 41).

Intestinal spirochetes have been classified on the basis of hemolytic, antigenic, and biochemical characteristics. RFLP fingerprinting of the PF *flaA1* gene of intestinal spirochetes did not provide subspecies differentiation, as reported by researchers using pulsed-field gel electrophoresis (1) and other genomic methods (5, 6, 21, 24, 26, 27, 31, 35, 36, 38, 41–43). These methods are useful for epidemiological tracing of colonic spirochetal infections within geographical locations and indirectly support the designation of existing species of *Serpulina* (9, 22, 40, 44).

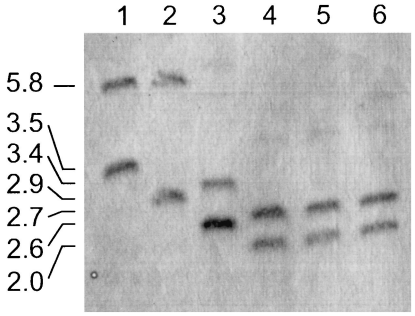

FIG. 6. Hybridization of the DIG-labeled *S. hyodysenteriae* isolate B78 PF *flaA1* probe with *Eco*RV-digested chromosomal DNA from reference and field isolates of nonclassified porcine intestinal spirochetes. Lane 1, *S. hyodysenteriae* isolate B78; lane 2, *S. innocens* isolate B256; lane 3, *S. pilosicoli* isolate P43/6/78; lane 4, "*S. intermedius*" isolate AN26:93; lane 5, "*S. intermedius*" isolate UNL-2; lane 6, "*S. intermedius*" isolate 889. The apparent molecular sizes are indicated at the left in kilobase pairs.

RFLP fingerprinting of the PF *flaA1* gene of intestinal spirochetes revealed variations among *Serpulina* species and *B. aalborgi*. The RFLP fingerprint patterns produced with the PF *flaA1* gene probe were distinct for *S. hyodysenteriae*, *S. innocens*, and *S. pilosicoli* and also were consistent with the provisional taxonomic groups, "*S. intermedius*" and "*S. murdochii*." The results of RFLP fingerprinting of the PF *flaA1* gene correlated well with previously defined phenotypic and genotypic classifications of intestinal spirochetes; there was complete agreement with accepted genera and species divisions (9, 20, 40, 44) and with classifications based on biochemical analyses (12, 26, 33, 36, 37), ribotyping (21, 42), MEE (26, 28), and 16S rRNA sequencing (12, 33, 36, 41). This relatively simple method should allow provisional identification of unknown intestinal spirochetes with a single DIG-labeled probe without the use of hazardous radioisotopes. The DIG-labeled probe was stable for 2 months at 4°C and may be stored for up to 1 year at −20°C (4), allowing more reproducible hybridization studies.

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