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Genomic Fingerprinting of "*Haemophilus somnus*" Isolates by Using a Random-Amplified Polymorphic DNA Assay

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The random-amplified polymorphic DNA (RAPD) assay was used to generate DNA fingerprints for 16 isolates of "*Haemophilus somnus*," and one isolate each of "*Haemophilus agni*," "*Histophilus ovis*," "*Actinobacillus seminis*," *Pasteurella haemolytica*, and *Escherichia coli*. The RAPD assay differentiated among "*H. somnus*" isolates, which shared similarity coefficients of 0.46 to 1.00 on the basis of pairwise comparisons of RAPD markers produced with nine random decamer primers. Three virulent encephalitic "*H. somnus*" isolates exhibited identical banding patterns, suggesting a common clonal ancestry. The RAPD assay clearly distinguished between the "*H. somnus*"-"*H. agni*"-"*H. ovis*" group and the other bacterial species tested. The results of the present study suggest that DNA fingerprinting of "*H. somnus*" isolates by the RAPD assay could be valuable in revealing subspecific divisions within this largely unexplored species.

"*Haemophilus somnus*" bacteria are gram-negative pleomorphic bovine pathogens which are carried asymptomatically in the healthy bovine urogenital and upper respiratory tracts. "*H. somnus*" is associated with a variety of bovine diseases: bronchopneumonia; septicaemic diseases, including thrombotic meningoencephalitis, myocardial abscessation, and arthritis; mastitis; and reproductive disorders, including inflammatory female genital disease, abortion, and infertility (11, 13, 18).

"*H. somnus*" is misplaced within the genus *Haemophilus* (15); although numerical-taxonomy and DNA-DNA and DNA-RNA hybridization studies indicate that "*H. somnus*" and ovine-derived "*Haemophilus agni*" and "*Histophilus ovis*" constitute a single species (5, 22, 25, 26), a definitive description of the species and designation of a type strain have not yet been attained. This is because a limited number of isolates have been employed for many of the phenetic tests and also because this group of isolates exhibits extensive variability in their morphology, biochemistry, and serology (1, 3, 8, 16, 24, 25). Consequently, exploration of subspecific relationships among "*H. somnus*" isolates has not been extensive. At least 15 different serotypes of "*H. somnus*" have been identified previously (3, 24), although the number of investigations to date has been limited. Multilocus enzyme electrophoresis has not been undertaken with these organisms. Plasmid typing and restriction endonuclease fingerprinting have been used to differentiate among a small number of isolates of "*H. somnus*," "*H. agni*," and "*H. ovis*" (16, 21).

Recently, a novel DNA fingerprinting technique based on the polymerase chain reaction (PCR) was reported by Williams et al. (29) and Welsh and McClelland (27). This technique, known as random-amplified polymorphic DNA (RAPD) assay, is based on the use of single arbitrary primers in a polymerase chain reaction (PCR) of low stringency to amplify segments of the genome. The resulting amplified fragments function as polymorphisms for DNA fingerprinting and construction of genetic maps. In the present study, we investigated the potential of the RAPD assay to differentiate among isolates of "*H. somnus*." The resulting RAPD fingerprints were also compared with the RAPD fingerprints of "*H. agni*," "*H. ovis*," and other closely and distantly related species.

(This work will form part of a thesis to be presented to the University of Guelph by L. E. Myers in partial fulfillment of the requirements for an M.S. degree.)

MATERIALS AND METHODS

Bacterial isolates. The "*H. somnus*" isolates used in this study were from a collection obtained from independent sources throughout Southern Ontario over a 10-year period. Bacterial isolates are described in Table 1; for simplicity, "*H. somnus*" isolates have been assigned code names on the basis of their virulence and tissue of origin. All isolates except *Escherichia coli* were propagated on brain heart infusion agar (Difco Laboratories, Detroit, Mich.) supplemented with 7% bovine blood, 1% yeast extract, and 1 μg of thiamine monophosphate per ml (25); *E. coli* was grown on Luria-Bertani agar (23). For each preparation of total cellular DNA, cultures were grown, at 37°C in 5% CO_2, to late-log phase in broth containing 3.7% brain heart infusion (Difco), 0.1% starch, 0.1% Na-L-aspartate, 0.1% Tris, and 1 μg of thiamine monophosphate per ml (25).

DNA isolation. DNA was isolated as previously described (20), with a few modifications. Bacterial cells were pelleted by centrifugation at 11,000 × *g* for 10 min, washed once in 100 mM Tris-Cl (pH 8.0)–150 mM NaCl–2 mM EDTA (TBSE), and resuspended in 1 ml of TBSE. *E. coli* cells were treated with 1 mg of lysozyme (Sigma Chemical Co., St. Louis, Mo.) per ml for 10 min on ice. Bacterial cells were lysed by the addition of sodium dodecyl sulfate to a final concentration of 0.5% in the presence of 25 mM EDTA. RNase (Boehringer Mannheim Canada, Laval, Québec, Canada) was added to a final concentration of 50 μg/ml, and the mixture was incubated for 30 min at 37°C. Proteinase K (Boehringer Mannheim Canada) was added to a final concentration of 50 μg/ml, and the mixture was incubated for a further 30 min at 37°C. A series of phenol-chloroform extractions were performed. A final extraction with chloroform alone aided in the removal of trace phenol, along with

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TABLE 1. Bacterial isolates

Taxon (code name)	Isolate	Geographical origin[a] (date of isolation)	Reference or source
"*H. somnus*" (VE-1)	43826	Embro (1978)	6
"*H. somnus*" (VE-2)	51372	Georgetown (1979)	12
"*H. somnus*" (VE-3)	91-1	Bruce County (1980)	12
"*H. somnus*" (VR-1)	70986	Paisley (1982)	9
"*H. somnus*" (VR-2)	37-1	Bruce County (1978)	Stephens[b]
"*H. somnus*" (VG-1)	88159	Kitchener[c] (1988)	19
"*H. somnus*" (VG-2)	8711337	Kitchener[c] (1987)	19
"*H. somnus*" (VG-3)	885104	Gads Hill (1988)	19
"*H. somnus*" (AG-1)	87121531	Kitchener[c] (1987)	19
"*H. somnus*" (AG-2)	S20	Woodstock (1980)	12
"*H. somnus*" (AG-3)	26-16	Kitchener[c] (1980)	21
"*H. somnus*" (AG-4)	S70	Guelph (1980)	12
"*H. somnus*" (AG-5)	88541	Denfield (1988)	19
"*H. somnus*" (AG-6)	88546	Alisa Craig (1988)	19
"*H. somnus*" (AG-7)	8851018	Thamesford (1988)	19
"*H. somnus*" (AG-8)	885514	Dorchester (1988)	19
"*H. ovis*"	43803	Guelph (1978)	21
"*H. agni*"	1344[d]	United States	21
"*A. seminis*"	ATCC[e] 15768	Australia (1959)	6
P. haemolytica	Serotype 1[f]	Canada	4
E. coli	HB101		10

[a] "*H. somnus*" isolates and "*H. ovis*" 43803 were obtained from independent sources throughout Southern Ontario, Canada.
[b] L. R. Stephens (present address, Veterinary Research Institute, 475 Mickleham Road, Attwood, Victoria, Australia).
[c] Collected at independent times from an abattoir (J. M. Schneider's Inc., Kitchener, Ontario, Canada); original geographic origin unknown.
[d] From E. L. Biberstein (present address, Department of Veterinary Microbiology and Immunology, University of California, Davis, Davis, Calif.).
[e] ATCC, American Type Culture Collection, Rockville, Md.
[f] From P. E. Shewen (present address, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada).

the pigmented protein material. After the addition of sodium acetate to a final concentration of 0.3 M, the mixture was overlaid with 1 volume of cold (−20°C) isopropanol, and the DNA was spooled onto a glass rod. The DNA was washed briefly in 70 and 90% ethanol, dried, and resuspended in 500 μl of 10 mM Tris-Cl (pH 8.0)–1 mM EDTA. Estimates of DNA concentration were obtained by A_{260} measurements (23). This extraction procedure yielded spoolable, high-molecular-weight DNA of good quality, as assessed by electrophoresis on a 0.7% agarose gel in 40 mM Tris acetate–2 mM EDTA (pH 8.5) (TAE buffer) and staining with 0.5 μg of ethidium bromide per ml.

PCR amplification. Primers were obtained as part of a series of random oligonucleotides produced at the Biotechnology Laboratory and Department of Forest Sciences, University of British Columbia, Vancouver, British Columbia, Canada. The nucleotide sequences and GC contents of the primers are listed in Table 2. The following reagents were added in a 25-μl reaction volume and overlaid with 20 μl of mineral oil: 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin (wt/vol), 0.1% Triton X-100, 2% formamide, 100 μM each nucleotide (Pharmacia LKB Biotechnology, Baie d'Urfé, Québec, Canada), 0.2 μM each primer, 10 ng of template DNA, and 2.0 U of *Taq* I DNA polymerase (Promega, Madison, Wis.). Following an initial denaturation of 2 min at 94°C, the PCR was carried out for 45 cycles in a Perkin-Elmer Cetus (Norwalk, Conn.) TC-1 Thermal Cycler programmed for 1 min at 94°C, 1 min at 40°C, and 2 min at 72°C. The PCR was followed by a final primer extension-polymerization of 10 min at 72°C. Negative controls were prepared as described above, with no addition of template DNA.

Amplified DNA was electrophoresed on 1.4% agarose gels in TAE buffer and stained with 0.5 μg of ethidium bromide per ml. The gels were photographed under UV illumination with a red filter and Polaroid (Cambridge, Mass.) type 57 film. Promega 2-kb pGEM fragments were used as molecular size markers.

Calculation of similarity coefficients. Pairwise comparisons of amplified DNA fragments generated by different primers among isolates of "*H. somnus*" and "*H. ovis*" 43803 were made. The similarity coefficient (F) between each pair of isolates was calculated by the formula described by Gabriel et al. (7): $F = (n_{xy} + n_{yx})/(n_x + n_y)$, where n_{xy} is the number of major amplified DNA fragments in isolate X matching any major or minor amplified DNA fragment in isolate Y, n_{yx} is the number of major amplified DNA fragments in isolate Y matching any major or minor amplified DNA fragment in isolate X, and n_x and n_y are the numbers of major amplified DNA fragments in isolates X and Y, respectively.

RESULTS

Amplification of DNA of "*H. somnus*" isolates. Sixteen different decamer primers were tested in the RAPD assay; the procedure generated RAPD fingerprints containing between 0 and 14 amplified DNA segments for each isolate (Fig. 1 and 2). Primer 124 failed to produce amplification products with any of the "*H. somnus*" isolates. Primers 125 and 135 produced background in control wells which contained no DNA, and the corresponding RAPD fingerprints were not used for comparison of any isolates. Background amplification was not observed in the negative control wells for any other primers used, and the inclusion of 2% formamide in the reaction mixture appeared to aid in minimizing background. Repeated amplifications generated similar RAPD fingerprints even with the use of different DNA preparations from the same isolates and in reaction mixtures in which DNAs from different isolates were mixed (Fig. 3 and 4). However, the intensities of the amplified segments,

TABLE 2. Nucleotide sequences and summary evaluation of primers

Primer	Sequence	GC content (%)	Analyzed[a]	Primer evaluation based on PCR with "H. somnus" and "H. ovis" isolates
112	5′ GCT TGT GAA C 3′	50	Yes	Informative fingerprints[b]
117	5′ TTA GCG GTC T 3′	50	Yes	Less-informative fingerprints[c]
124	5′ ACT CGA AGT C 3′	50	No	No amplification
125	5′ CGC GTT GAG G 3′	70	No	Background in negative control wells
126	5′ CTT TCG TGC T 3′	50	Yes	Informative fingerprints[b]
127	5′ ATC TGG CAG C 3′	60	Yes	Informative fingerprints[b]
128	5′ GCA TAT TCC G 3′	60	Yes	Informative fingerprints[b]
129	5′ GCG GTA TAG T 3′	50	Yes	Informative fingerprints[b]
131	5′ GAA ACA GCG T 3′	50	Yes	Less-informative fingerprints[c]
132	5′ AGG GAT CTC C 3′	60	No	Patterns difficult to analyze
133	5′ GGA AAC CTC T 3′	50	Yes	Less-informative fingerprints[c]
134	5′ AAC ACA CGA G 3′	50	No	Patterns difficult to analyze
135	5′ AAG CTG CGA G 3′	60	No	Background in negative control wells
136	5′ TAC GTC TTG C 3′	50	No	Patterns difficult to analyze
137	5′ GGT CTC TCC C 3′	70	Yes	Informative fingerprints[b]
138	5′ GCT TCC CCT T 3′	60	No	Patterns difficult to analyze

[a] Indicates whether RAPD fingerprints generated by these primers were used in the calculation of similarity coefficients among "*H. somnus*" isolates and "*H. ovis*" 43803.

[b] Informative primers differentiated between 125 and 129 of 136 pairs of "*H. somnus*" and "*H. ovis*" isolates or between 97 and 103 of 105 isolate pairs if any two of the encephalitic isolates VE-1, VE-2, or VE-3, which produced identical RAPD fingerprints, were excluded from the pairwise comparison.

[c] Less-informative primers differentiated between fewer than 120 of 136 "*H. somnus*"-"*H. ovis*" isolate pairs or fewer than 94 of 105 isolate pairs, with any two of the encephalitic isolates VE-1, VE-2, and VE-3 excluded from the pairwise comparison.

especially of the minor bands, varied with repeated amplifications of DNA from the same isolate.

Differentiation of isolates and species. Depending on the primer used, the RAPD fingerprints of "*H. somnus*" DNAs exhibited a range of banding profiles, with some primers yielding more-informative arrays of amplified fragments than others. Four of the primers (Table 2) produced patterns containing multiple faint fragments that were difficult to analyze; these fingerprints were not used in the calculation of similarity coefficients among "*H. somnus*" and "*H. ovis*" isolates. As indicated in Table 2, nine of the primers produced patterns suitable for use in the determination of correlation coefficients. Six of the nine primers produced fingerprints containing a variety of polymorphic sites (Fig. 1) and differentiated between at least 92% or more of "*H. somnus*" isolate pairs (Table 2). The other three primers resulted in less-informative arrays of bands that were conserved among all isolates (Fig. 2). Figure 5 presents several examples of amplified fragments, which are marked to indicate which bands were considered to be major or minor in the comparison of isolate pairs. Similarity coefficients for each pair of isolates, on the basis of comparisons of RAPD markers generated with all nine primers, are presented in Table 3. The values of the similarity coefficients for "*H. somnus*" isolates ranged from 0.46 to 1.00. With the exception of a few minor bands, the RAPD fingerprints of the three virulent encephalitic (VE) isolates (VE-1, VE-2, and VE-3) were virtually identical. The encephalitic isolates shared higher correlation coefficients with virulent respiratory (VR) isolate VR-2 (0.83 to 0.85) and virulent genital (VG) isolate VG-2 (0.78 to 0.80) than with all other "*H. somnus*" isolates tested (0.46 to 0.70). Isolate VR-1 shared higher similarity coefficients (0.72 to 0.75) with avirulent genital (AG) isolates AG-1, AG-3, and AG-5 than with isolate VR-2 (0.46). The similarity coefficients for all genital isolates ranged from 0.46 (AG-6 and AG-8) to 0.87 (AG-1 and AG-3). "*H. ovis*" 43803

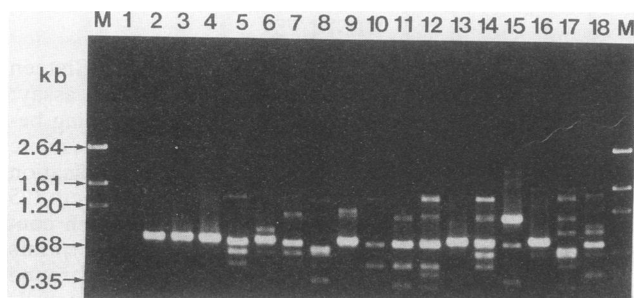

FIG. 1. RAPD fingerprints of "*H. somnus*" isolates and "*H. ovis*" 43803 with primer 129. "*H. somnus*" isolates are VE-1, VE-2, and VE-3 (lanes 2 to 4, respectively); VR-1 and VR-2 (lanes 5 and 6, respectively); VG-1, VG-2, and VG-3 (lanes 7 to 9, respectively); and AG-1, AG-2, AG-3, AG-4, AG-5, AG-6, AG-7, and AG-8 (lanes 10 to 17, respectively). Lane 18 is "*H. ovis*" 43803; lane 1 is the negative control with no template DNA. Lanes M are molecular size markers (in kilobases).

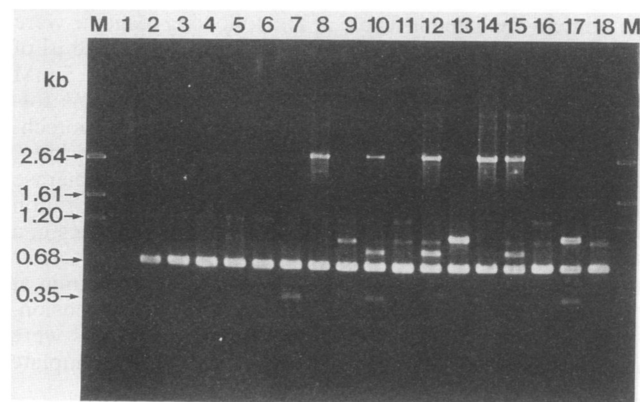

FIG. 2. RAPD fingerprints of "*H. somnus*" isolates and "*H. ovis*" 43803 with primer 131. Isolates, negative control, and molecular size markers are as described in the legend to Fig. 1.

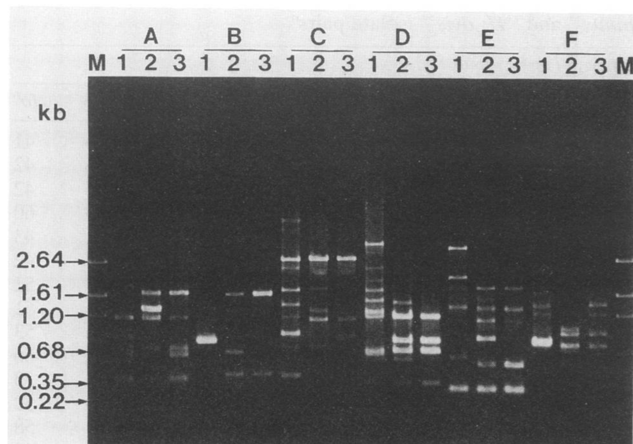

FIG. 3. RAPD fingerprints of isolates of "*H. somnus*," "*H. agni*," and "*H. ovis*." The following primers were used: 112 (A), 117 (B), 126 (C), 127 (D), 128 (E), and 129 (F). All panels contain "*H. agni*" 1344 in lane 2 and "*H. ovis*" 43803 in lane 3. Lane 1 of each panel contains the following "*H. somnus*" DNAs: VR-1 (A), VE-1 and VG-3 (B), VR-1 and VG-1 (C), VE-1 and AG-4 (D), VE-1 and AG-6 (E), and VE-1 and VR-1 (F). Lanes M are molecular size markers (in kilobases).

shared coefficients of 0.41 to 0.68 with "*H. somnus*" isolates (Table 3) and 0.74 with "*H. agni*" 1344 (results not shown).

All of the primers listed in Table 2, with the exception of primers 124, 125, 135, and 136, were used to amplify DNA from "*Actinobacillus seminis*" ATCC 15768, *Pasteurella haemolytica* serotype 1, and *E. coli* HB101. For convenience in comparing these fingerprints to "*H. somnus*" fingerprints on the same gel, DNAs from different "*H. somnus*" isolates were mixed to reproduce the majority of RAPD markers previously observed with all "*H. somnus*" isolate-primer combinations. The RAPD fingerprints generated were unique for each species, and none of these species had any RAPD markers in common with "*H. somnus*" isolates (Fig. 4).

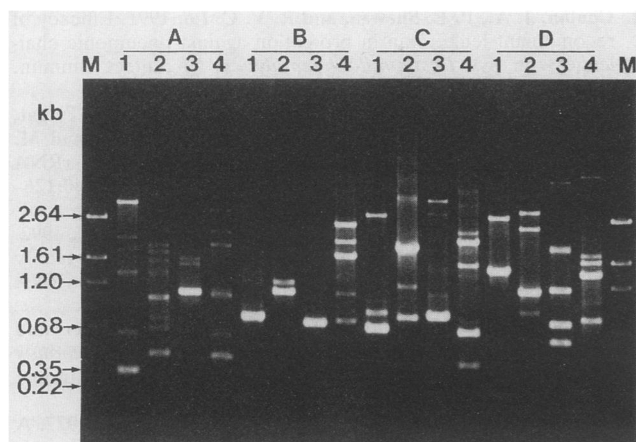

FIG. 4. RAPD fingerprints of isolates of "*H. somnus*," "*A. seminis*," *P. haemolytica*, and *E. coli*. The primers used were 128 (A), 129 (B), 131 (C), and 132 (D). All panels contain "*A. seminis*" ATCC 15768 in lane 2, *P. haemolytica* serotype 1 in lane 3, and *E. coli* HB101 in lane 4. Lane 1 of each panel contains the following "*H. somnus*" isolate DNAs: VE-1 and AG-6 (A), AG-1 and VR-1 (B and D), and VE-1 and AG-1 (C). Molecular size markers (M) are indicated in kilobases.

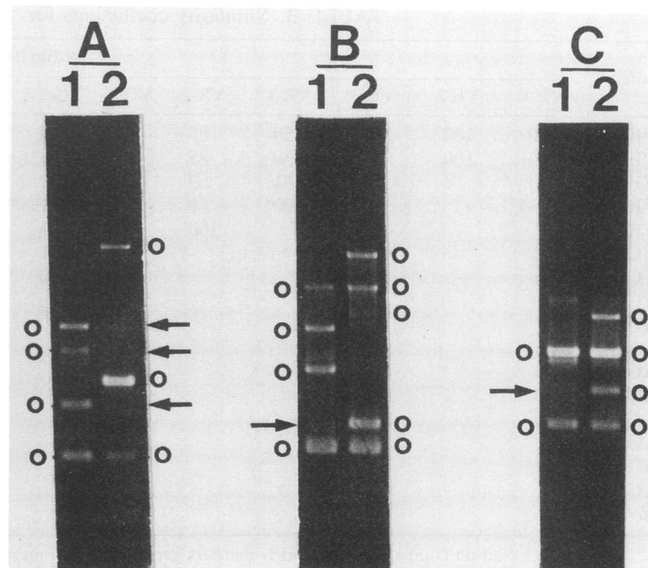

FIG. 5. Examples of RAPD fingerprints marked to indicate major fragments (open circles) or minor fragments (arrows) used in the calculation of similarity coefficients between the following "*H. somnus*" isolate pairs. The primers used were 126 (A), 127 (B), and 137 (C). Lanes 1 and 2, respectively, contain the following "*H. somnus*" isolate DNAs: VE-3 and VR-1 (A), VR-2 and VG-1 (B), and VE-3 and VR-1 (C). Each additional unmarked and visible band was included in the analysis as a minor fragment if it matched a major fragment in another isolate; otherwise, it was excluded from the analysis.

DISCUSSION

Although "*H. somnus*" has been recognized during the last few decades as an etiological agent in a complex of bovine diseases, the taxonomy of this organism remains unresolved, and efforts to type isolates to determine subspecific relationships have not been extensive. Recently, the RAPD assay has been utilized as a simple and rapid alternative method of DNA fingerprinting and has been used successfully to differentiate among strains of *Streptococcus pyogenes*, *Staphylococcus* spp. (27), and *Lactococcus lactis* (2). The technique also was reported to be useful in subtyping strains of *Streptococcus uberis* (14). The results reported in the present paper indicate that the RAPD assay can be used to differentiate among isolates of "*H. somnus*" and suggest that the technique should be useful for distinguishing the "*H. somnus*"-"*H. agni*"-"*H. ovis*" group from other related bacterial species.

The production of genetic markers through the RAPD assay has several advantages over other DNA fingerprinting techniques. Analysis of patterns is much simpler than analysis of the complex number of bands generated by restriction endonuclease fingerprinting of total cellular DNA. The RAPD assay is faster and less labor intensive than Southern blot hybridizations for restriction fragment length polymorphism analysis and does not require specific nucleotide sequence information. Previous data suggest that RAPD results are comparable and may offer a degree of sensitivity higher than those of other DNA fingerprinting techniques (14, 29).

TABLE 3. Similarity coefficients for "*H. somnus*" and "*H. ovis*" isolate pairs[a]

Isolate	Similarity coefficient (10^2)																
	VE-1	VE-2	VE-3	VR-1	VR-2	VG-1	VG-2	VG-3	AG-1	AG-2	AG-3	AG-4	AG-5	AG-6	AG-7	AG-8	*Ho*[b]
VE-1	100	100	100	59	85	60	78	70	60	57	52	50	49	53	64	46	41
VE-2		100	100	59	85	66	78	70	60	57	52	50	49	53	65	46	42
VE-3			100	61	83	60	80	70	60	58	52	50	49	53	65	46	42
VR-1				100	46	63	71	52	72	60	73	59	75	63	64	63	60
VR-2					100	67	56	58	54	51	52	54	60	53	67	61	43
VG-1						100	62	57	60	53	61	59	68	54	66	57	42
VG-2							100	66	60	69	64	51	68	59	73	59	51
VG-3								100	61	74	58	55	54	61	64	55	51
AG-1									100	61	87	63	66	74	68	56	53
AG-2										100	66	55	61	60	70	61	66
AG-3											100	49	64	76	62	59	53
AG-4												100	60	48	66	70	52
AG-5													100	58	68	64	58
AG-6														100	64	46	46
AG-7															100	68	68
AG-8																100	60
Ho[b]																	100

[a] Results are based on comparisons of RAPD markers generated by primers 112, 117, 126, 127, 128, 129, 131, 133, and 137.
[b] *Ho*, "*H. ovis*" 43803.

Genomic typing methods such as restriction endonuclease fingerprinting have been useful in revealing bacterial clonal characteristics that subsequently may be related to virulence among bacterial strains (17). A common clonal ancestry among the three VE "*H. somnus*" isolates, all originating from different regions of Southern Ontario, Canada, is suggested by the close similarity of their RAPD fingerprints and is substantiated by the fact that the same isolates have identical restriction endonuclease fingerprints (results not shown). Testing of a larger number of isolates from the "*H. somnus*"-"*H. agni*"-"*H. ovis*" group may reveal whether this putative clonal group is always associated with a specific target tissue or host.

The similarity coefficients of "*H. somnus*" isolate pairs in this study ranged from 0.46 to 1.00, on the basis of the use of nine primers; similar data generated by the RAPD assay could be used to cluster isolates into subspecific groups for epidemiological studies. For such an application, screening a sufficiently large number of primers is important to ensure that the analysis is based on a statistically adequate number of informative primers (i.e., primers that reveal a variety of unique polymorphic sites among isolates). Alternatively, a relatively small number of primers could be used in pairwise combinations to generate a large number of RAPD fingerprints, in which at least 50% of the resulting polymorphisms are unique to each primer pair, as reported by Welsh and McClelland (28). The use of primer pairs should increase the potential for finding new polymorphisms among all isolates.

While most of the primers generated at least one RAPD marker that was amplified consistently in all "*H. somnus*," "*H. ovis*" and "*H. agni*" isolates tested (e.g., primer 131 [Fig. 2]), 12 primers clearly differentiated between isolates of this species and isolates of distantly related species of *P. haemolytica* and *E. coli*. Recent sequencing of 16S rRNA has indicated that "*A. seminis*" and "*H. somnus*" are closely related species (6); the primers used in the present work also clearly distinguished between isolates belonging to these two species. It is likely that the RAPD assay should prove to be useful for differentiating "*H. somnus*" from additional species which subsequently are classified as "close" relatives, as taxonomic characterization of members of the family *Pasteurellaceae* proceeds.

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REFERENCES

1. **Broom, A. K., and P. H. A. Sneath.** 1981. Numerical taxonomy of *Haemophilus*. J. Gen. Microbiol. **126:**123–149.
2. **Cancilla, M. R., I. B. Powell, A. J. Hillier, and B. E. Davidson.** 1992. Rapid genomic fingerprinting of *Lactococcus lactis* strains by arbitrarily primed polymerase chain reaction with ^{32}P and fluorescent labels. Appl. Environ. Microbiol. **58:**1772–1775.
3. **Canto, G. J., and E. L. Biberstein.** 1982. Serological diversity in *Haemophilus somnus*. J. Clin. Microbiol. **15:**1009–1015.
4. **Conlon, J. A., P. E. Shewen, and R. Y. C. Lo.** 1991. Efficacy of recombinant leukotoxin in protection against pneumonic challenge with live *Pasteurella haemolytica* A1. Infect. Immun. **59:**587–591.
5. **De Ley, J., W. Mannheim, R. Mutters, K. Piechulla, R. Tytgat, P. Segers, M. Bisgaard, W. Frederiksen, K.-H. Hinz, and M. Vanhoucke.** 1990. Inter- and intrafamilial similarities of rRNA cistrons of the *Pasteurellaceae*. Int. J. Syst. Bacteriol. **40:**126–137.
6. **Dewhirst, F. E., B. J. Paster, I. Olsen, and G. J. Fraser.** 1992. Phylogeny of 54 representative strains of species in the family *Pasteurellaceae* as determined by comparison of 16S rRNA sequences. J. Bacteriol. **174:**2002–2013.
7. **Gabriel, D. W., J. E. Hunter, M. T. Kingsley, J. W. Miller, and G. R. Lazo.** 1988. Clonal population structure of *Xanthomonas campestris* and genetic diversity among citrus canker strains. Mol. Plant-Microbe Interact. **1:**59–65.
8. **Garcia-Delgado, G. A., P. B. Little, and D. A. Barnum.** 1977. A comparison of various *Haemophilus somnus* strains. Can. J. Comp. Med. **41:**380–388.
9. **Groom, S. C., P. B. Little, and S. Rosendal.** 1988. Virulence differences among three strains of *Haemophilus somnus* following intratracheal inoculation of calves. Can. J. Vet. Res. **52:**349–354.
10. **Hanahan, D. J., J. Jesse, and F. R. Bloom.** 1991. Plasmid transformation of *Escherichia coli* and other bacteria. Methods Enzymol. **204:**63–113.

11. **Harris, F. W., and E. D. Janzen.** 1989. The *Haemophilus somnus* disease complex (hemophilosis): a review. Can. Vet. J. **30:**816–822.
12. **Humphrey, J. D.** 1982. Ph.D. thesis. University of Guelph, Guelph, Ontario, Canada.
13. **Humphrey, J. D., and L. R. Stephens.** 1983. "*Haemophilus somnus*": a review. Vet. Bull. **53:**987–1004.
14. **Jayarao, B. M., B. J. Bassam, G. Caetano-Anollés, P. M. Gresshoff, and S. P. Oliver.** 1992. Subtyping of *Streptococcus uberis* by DNA amplification fingerprinting. J. Clin. Microbiol. **30:**1347–1350.
15. **Kilian, M., and E. L. Biberstein.** 1984. Genus II. *Haemophilus* Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1917, 561[AL], p. 558–569. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
16. **Kirkham, C., E. L. Biberstein, and R. B. LeFebvre.** 1989. Evidence of host-specific subgroups among "*Histophilus ovis*" isolates. Int. J. Syst. Bacteriol. **39:**236–239.
17. **Kristiansen, B. E., B. Bjorvatn, V. Lund, B. Lindqvist, and E. Holten.** 1984. Differentiation of B15 strains of *Neisseria meningitidis* by DNA restriction endonuclease fingerprinting. J. Infect. Dis. **150:**672–677.
18. **Kwiecien, J. M., and P. B. Little.** 1991. *Haemophilus somnus* and reproductive disease in the cow: a review. Can. Vet. J. **32:**595–601.
19. **Kwiecien, J. M., and P. B. Little.** 1992. Isolation of pathogenic strains of *Haemophilus somnus* from the female reproductive tract. Can. J. Vet. Res. **56:**127–134.
20. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. **3:**208–218.
21. **McGillivery, D. J., J. J. Webber, and H. F. Dean.** 1986. Characterization of *Histophilus ovis* and related organisms by restriction endonuclease analysis. Aust. Vet. J. **63:**389–393.
22. **Piechulla, K., R. Mutters, S. Burbach, R. Klussmeier, S. Pohl, and W. Mannheim.** 1986. Deoxyribonucleic acid relationships of "*Histophilus ovis/Haemophilus somnus*," *Haemophilus haemoglobinophilus*, and "*Actinobacillus seminis*". Int. J. Syst. Bacteriol. **36:**1–7.
23. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
24. **Stephens, L. R., R. Aukema, and L. J. L. Murray.** 1987. Antigenic heterogeneity of *Haemophilus somnus*. Aust. Vet. J. **64:**113.
25. **Stephens, L. R., J. D. Humphrey, P. B. Little, and D. A. Barnum.** 1983. Morphological, biochemical, antigenic and cytochemical relationships among *Haemophilus somnus*, *Haemophilus agni*, *Haemophilus haemoglobinophilus*, *Histophilus ovis*, and *Actinobacillus seminis*. J. Clin. Microbiol. **17:**728–737.
26. **Walker, R. L., E. L. Biberstein, R. F. Pritchett, and C. Kirkham.** 1985. Deoxyribonucleic acid relatedness among *Haemophilus somnus*, *Haemophilus agni*, *Histophilus ovis*, *Actinobacillus seminis*, and *Haemophilus influenzae*. Int. J. Syst. Bacteriol. **35:**46–49.
27. **Welsh, J., and M. McClelland.** 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. **18:**7213–7218.
28. **Welsh, J., and M. McClelland.** 1991. Genomic fingerprinting using arbitrarily primed PCR and a matrix of pairwise combinations of primers. Nucleic Acids Res. **19:**5275–5279.
29. **Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey.** 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18:**6531–6535.