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Comparison of DNA Fingerprints and Somatic Serotypes of Serogroup B and E *Pasteurella multocida* Isolates

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The DNA fingerprint profiles and somatic serotypes of 71 *Pasteurella multocida* capsule serogroup B isolates, 13 capsule serogroup E isolates, and 16 somatic reference serotype strains were compared. Each of the 16 reference somatic serotypes had a unique DNA fingerprint profile with the *Hha*I restriction endonuclease. Fifty-four serogroup B isolates (isolated from classical cases of hemorrhagic septicemia) reacted with somatic serotype 2 or 5 antiserum and had DNA fingerprint profiles which resembled that of the serotype 2 reference strain. Seven DNA fingerprint profiles were found among 16 serogroup B strains representing other somatic serotypes. The DNA fingerprints of these isolates were different from the fingerprints of the 16 somatic reference serotype strains. All 13 serogroup E isolates had identical somatic serotypes and identical DNA fingerprint profiles when the *Hha*I endonuclease was used. The *Hha*I fingerprint profile of the serogroup E isolates did not match any fingerprint profile of the reference somatic serotype strains. Following DNA profiling with the *Hha*I endonuclease, the 13 serogroup E isolates were differentiated sequentially with *Hpa*II restriction endonuclease. A descriptive identification epithet for *P. multocida* isolates was constructed. The descriptive epithet consists of serologic identification and sequential DNA profiles with restriction endonucleases *Hha*I and *Hpa*II, respectively. DNA fingerprinting of *P. multocida* is a precise characterization method. In conjunction with serologic typing, it can further classify *P. multocida* isolates for epidemiologic studies.

Pasteurella multocida is characterized serologically by capsule serogroup with passive hemagglutination tests (2, 11) and somatic antigen type with gel diffusion precipitin tests (4). Either or both of these tests have been used to compare isolates in toxin studies (9), lipopolysaccharide analysis (8, 10), and surveys of the organism in various domestic and wildlife species (1, 6, 7, 12).

Five capsule serogroups (designated A, B, D, E, and F) of *P. multocida* have been described (2, 11). Among these, serogroups A and D are commonly isolated in the United States, but occasionally organisms of serogroups B and F are found. In addition to the capsule serogroups, 16 major somatic serotypes (identified as 1 through 16) are recognized. *P. multocida* isolates that express multiple major somatic antigens are also frequently encountered. Lipopolysaccharides are the antigens that determine somatic type specificity. Recent chemical and antigenic analyses of *P. multocida* lipopolysaccharides indicate that differences between serotypes 2 and 5 are minor and may not be sufficient to warrant their separation (8). Various somatic serotypes occur among the different capsule serogroups, and current serological nomenclature designates both of these features. For example, *P. multocida* of capsule serogroup A and somatic serotype 3 is described as A:3.

It is well known that *P. multocida* with certain antigenic compositions can be associated with specific diseases in animals. This is especially true for the B:2 and E:2 serotypes. These serotypes cause hemorrhagic septicemia, a devastating disease of cattle and buffalo in certain enzootic areas of Asia and Africa. Even though the B:2 serotype occurs primarily in Asia and Africa, it has been isolated from bison on occasion in the United States. The E:2 serotype has been isolated only from animals in Africa.

Serotyping and DNA fingerprinting are tools which can be used to resolve relationships among bacterial isolates. Serologic typing of both capsule and somatic antigens is an aid for diagnosis and recognition of the hemorrhagic septicemia-causing strains of *P. multocida*. Nevertheless, serologic characterization is limited and does not provide sufficient information for epidemiologic studies in which distinction among field and vaccine strains would be desirable. Avian field isolates and vaccine strains of *P. multocida* have been differentiated with DNA fingerprinting (5, 13). DNA fingerprinting, together with serologic typing, has been used to characterize porcine *P. multocida* for epidemiologic studies (3). Use of both DNA fingerprinting and serologic typing for characterization of the hemorrhagic septicemia strains of *P. multocida* has not been reported.

In this study, a rapid method for extracting DNA from *P. multocida* was developed. This method permits efficient processing of isolates for DNA fingerprinting. The DNA fingerprint profiles were used in conjunction with serologic typing to characterize serogroup B and E isolates. The DNA fingerprint profiles of all isolates that were generated with the *Hha*I endonuclease were compared with the *Hha*I endonuclease profiles of the 16 somatic serotype reference strains. Further differentiation of the E:2 isolates was accomplished by use of the *Hpa*II restriction endonuclease. The purpose of this study was to determine whether the use of serologic identification and systematic DNA fingerprinting with *Hha*I and *Hpa*II could resolve relationships among *P. multocida* isolates that could not be resolved with other methods.

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TABLE 1. Serotypes, DNA fingerprint profiles, and origins of *P. multocida* somatic reference serotype strains

Strain	Serotype[a]	DNA fingerprint profile (*Hha*I)	Source	Geographic origin
X-73	A:1	001	Chicken	Maryland
M-1404	B:2,5[b]	002	Bison	Wyoming
P-1059	A:3	003	Turkey	West Virginia
P-1662	A:4	004	Turkey	South Carolina
P-1702	A:5,2[c]	005	Turkey	Virginia
P-2192	—:6	006	Turkey	Texas
P-1997	—:7	007	Herring gull	New York
P-1581	—:8	008	Pine siskin	Massachusetts
P-2095	A:9	009	Turkey	Minnesota
P-2100	A:10	010	Turkey	Indiana
P-903	—:11	011	Swine	Maryland
P-1573	A:12	012	Human	Iowa
P-1591	—:13	013	Human	Iowa
P-2225	A:14	014	Cattle	Iowa
P-2237	—:15	015	Turkey	Iowa
P-2723	A:16	016	Turkey	Indiana

[a] The letter denotes the capsule serogroup, the number denotes the somatic serotype, — denotes a noncapsulated strain.
[b] Somatic serotype 2 reference strain.
[c] Somatic serotype 5 reference strain.

MATERIALS AND METHODS

Bacteria. One hundred *P. multocida* cultures were used in this study. Eighty-one isolates were from the culture collection of the National Animal Disease Center, Ames, Iowa; three field isolates and 16 reference somatic serotype strains of *P. multocida* were from the culture collection of the National Veterinary Services Laboratories, Ames, Iowa. The capsule groups, animal sources, and geographic origins of the 16 somatic reference strains are listed in Table 1. Seventy-one of these isolates were capsule serogroup B, and 13 were capsule serogroup E. These isolates are listed in Tables 2 and 3, respectively. For reference purposes hereafter, any serogroup B or E organisms containing the 2 or 5 somatic antigen are designated as classic hemorrhagic septicemia-causing *P. multocida* isolates.

Media and cultures. The cultures were initially grown on 5% bovine blood agar plates with incubation at 37°C for 18 to 24 h. Growth from a single colony was inoculated into a tube with a blood agar base slant and into a tube containing 5.0 ml of Trypticase soy broth with 1.25% tryptose (TST). All cultures were incubated at 37°C for 18 to 24 h. The blood agar base slant was used for subculturing to maintain stocks and inoculating a glucose starch agar plate to propagate cells for capsule and somatic antigen typing. The TST culture was used for DNA extraction.

A 1.5-ml aliquot of the 24-h TST broth culture was centrifuged at 16,000 × *g* for 4 min. The supernatant was discarded, and the pellet was suspended in 1.0 ml of 10 mM Tris–1 mM EDTA (TE; pH 8.0). The resulting mixture was recentrifuged as described above, and all but 50 μl of the supernatant was decanted; the residual supernatant and pellet were stored at −70°C until required.

Serologic typing. The somatic antigen composition of each isolate was determined by the method of Heddleston et al. (4). Capsule group typing was done as described previously (9).

Isolation of chromosomal DNA. Frozen pellets were thawed by addition of 350 μl of TE buffer and vortexed to resuspend the cells. One hundred fifty microliters of freshly prepared lysozyme solution (10 mg of lysozyme per ml of H_2O) was added to the suspension, and the mixture was placed on ice for 15 min to lyse the cells. After lysis, 40 μl of 10% sodium dodecyl sulfate solution in distilled H_2O was added and mixed for 1 min or until the suspension cleared. Eight microliters of RNase solution (100 mg/ml of H_2O) was added to the cleared suspension and mixed by repeated inversion of the tube for 1 min. Sixty microliters of proteinase K solution (20 mg/ml H_2O) was then added; the suspension was mixed by inversion, and the resulting mixture was incubated at 37°C for 30 min.

DNA was extracted by addition of 0.8 ml of phenol (pH 7.0) to a microcentrifuge tube of the above-described mixture. The tube was inverted vigorously until a white emulsion formed and then centrifuged at 16,000 × *g* for 1 min. After centrifugation, 600 μl of the aqueous phase was transferred to a clean microcentrifuge tube containing 150 μl of TE buffer. A 0.7-ml volume of a 1:1 mixture of phenol (pH 7.0) and chloroform-isoamyl alcohol (25:1, vol/vol) was added. The tube was vigorously inverted until a white emulsion formed and then centrifuged at 16,000 × *g* for 1 min. Six hundred microliters of the aqueous phase was transferred to a clean tube. Approximately 0.8 ml of chloroform-isoamyl alcohol (25:1) was added, and the tube was inverted several times before recentrifugation as before. Following centrifugation, 425 μl of the aqueous phase was transferred to a tube containing 75 μl of 3 M sodium acetate. The mixture was mixed briefly with a micropipet. Approximately 1.0 ml of ethanol (25°C) was added, and the tube was inverted five or six times prior to being placed on ice for 10 min. The precipitated DNA was pelleted by centrifugation for 15 min at 16,000 × *g*; the supernatant was decanted and discarded. The open tube of pelleted DNA was inverted on absorbent paper for 2 or 3 min, dried in a vacuum concentrator (Savant Instruments, Inc.) for approximately 30 min, and finally suspended in 50 μl of TE buffer.

Restriction endonuclease digestion, electrophoresis, and photography. Preliminary studies of restriction endonuclease digestion of *P. multocida* DNA were done with *Eco*RI, *Kpn*I, *Hin*dIII, and *Hha*I. Of these, *Hha*I yielded fingerprint profiles that were most easily and best distinguished. Therefore, all primary digestion studies were done with this endonuclease. Upon completion of serogroup E fingerprinting with *Hha*I, a second group of enzymes (*Alu*I, *Bgl*II, *Dra*I, *Dpn*I, *Eco*RV, *Hae*III, *Hinc*II, *Hin*fI, *Hpa*II, *Mbo*I, *Pst*I, *Rsa*I, and *Sma*I) were evaluated for the ability to differentiate these isolates. The *Hpa*II endonuclease yielded fingerprint profiles that were most easily distinguished. All secondary studies were done with *Hpa*II.

Digestion of the DNA with each restriction endonuclease (Bethesda Research Laboratories, Inc.) was done as recommended by the manufacturer. The reaction was stopped at 3 h by addition of 5.0 μl of stop mixture (0.25% bromophenol, 0.25% xylene cyanole, 25.0% Ficoll 400). TBE buffer (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA, pH 8.0) was used as an electrophoresis buffer. The digested DNA fragments were electrophoresed in a 0.7% agarose gel for 17 h at 60 V in an H-4 horizontal electrophoresis system (20 by 25 cm; Bethesda Research Laboratories, Inc.). The gel was stained with ethidium bromide and photographed with a Polaroid MP-34 camera using an Eastman Kodak 23A filter and UV illumination.

RESULTS

Comparison of DNA fingerprint profiles of reference somatic serotypes 1 through 16. Each of the 16 reference

TABLE 2. Serotypes, origins, and DNA fingerprint profiles of *P. multocida* capsule serogroup B isolates

Strain	Serotype	DNA fingerprint profile (*Hha*I)	Source	Geographic origin
P-3705	B:2,5	018	Buffalo	Sri Lanka
P-3708	B:2	021	NA[a]	Sri Lanka
P-3713	B:2,5	018	Cattle	Bangladesh
P-4258	B:2,5	019	Yak	China
P-4255	B:2,5	019	Buffalo	China
P-4651	B:2,5	018	Buffalo	India
P-4654	B:2,5	018	Buffalo	India
P-4656	B:2,5	018	Deer	India
P-4866	B:2	020	Buffalo	Malaysia
P-4869	B:2	020	Swine	Malaysia
P-4995	B:2	018	Swine	India
P-4999	B:2	018	Swine	India
P-5121	B:2,5	022	Cattle	United Kingdom
P-5140	B:2,5	018	Swine	India
P-5255	B:2,5	018	Cattle	India
P-5275	B:2,5	023	Cattle	Philippines
P-5303	B:2,5	018	Cattle	Zimbabwe
P-5304	B:2,5	018	Cattle	Zimbabwe
P-5305	B:2,5	018	Cattle	Zimbabwe
P-932	B:5	002	Bison	NA
P-4865	B:2, sl[b] 12	020	Swine	Malaysia
P-4867	B:2, sl 12	020	Swine	Malaysia
P-4868	B:2	020	Swine	Malaysia
P-4887	B:2, sl 12	018	Avian	Malaysia
P-4983	B:2	018	Cattle	Sri Lanka
P-4992	B:2	018	Swine	India
P-4993	B:2	018	Swine	India
P-4994	B:2	026	Swine	India
P-4996	B:2	027	Swine	India
P-4997	B:2	018	Swine	India
P-4998	B:2	024	Swine	India
P-5000	B:2	018	Swine	India
P-5001	B:2	018	Swine	India
P-5002	B:2,5	028	Swine	India
P-5003	B:2	018	Swine	India
P-5004	B:2	018	Swine	India
P-5005	B:2	028	Swine	India
P-5006	B:2	018	Swine	India
P-5007	B:2	027	Swine	India
P-5008	B:2	018	Swine	India
P-5009	B:2	018	Swine	India
P-5010	B:2	025	Swine	India
P-5011	B:2	018	Swine	India
P-5012	B:2	018	Swine	India
P-5013	B:2	018	Swine	India
P-5014	B:2	018	Swine	India
P-5015	B:2	029	Swine	India
314-1	B:2,5	018	NA	Philippines
314-2	B:2,5	018	NA	Philippines
314-3	B:—[c]	018	NA	Philippines
5041	B:2,5	020	Deer	Spain
5042	B:2,5	020	Deer	Spain
5043	B:2,5	020	Deer	Spain
5044	B:2,5	020	Deer	Spain
5045	B:2,5	020	Deer	Spain
P-5227	B:3,4	036	NA	United States
P-1458	B:3,4	032	Buffalo	United States
P-4674	B:3,4	033	Deer	United Kingdom
P-4675	B:3,4	033	Deer	United Kingdom
P-4676	B:3,4	033	Deer	United Kingdom
P-5225	B:3,4	036	Elk	United States
P-5226	B:3,4	036	Elk	United States
P-5231	B:3,4	036	Elk	United States
P-1203	B:3,4	031	NA	United States
P-1204	B:3,4	031	NA	United States
P-2105	B:4	034	Turkey	United States
P-2258	B:4	035	Turkey	United States
P-2104	B:4	034	Turkey	United States
P-5151	B:4, sl 12	035	Turkey	Netherlands
P-3270	B:1	030	Swan	United States
P-3269	B:1	030	Swan	United States

[a] NA, species or country of origin not available.
[b] sl, slight reaction detected.
[c] —, nonreactive against somatic antisera.

somatic serotypes of *P. multocida* produced a unique fingerprint profile upon digestion with *Hha*I; these were designated *Hha*I profiles 001 to 016 (Table 1; Fig. 1).

Comparison of isolates within capsule group B. Eight somatic serotypes (1; 2; 2, sl 12; 2,5; 5; 3,4; 4; and 4, sl 12) were identified among the capsule group B isolates (Table 2). One isolate (314-3) was nonreactive against any of the 16 somatic typing sera. Of the group B isolates, 54 with antigenic formula B:2; B:2, sl 12; or B:2,5 were from animals diagnosed as having hemorrhagic septicemia.

Among the group B isolates, 20 DNA fingerprint profiles were recognized with the *Hha*I endonuclease. Thirteen of these *Hha*I profiles (designated 002 and 018 to 029) were recognized (Fig. 2) among the classic hemorrhagic septicemia-causing *P. multocida* isolates (serotype B:2 or B:2,5). There were several instances in which these *P. multocida* isolates were serologically identical but had different DNA fingerprint profiles. Only isolate P-932 had a DNA fingerprint profile, *Hha*I 002 (Fig. 2, lane 2), that was identical to that of the reference somatic serotype 2 strain, M-1404 (Fig. 1, lane 2). The history of isolate P-932 is unclear, but it is believed to be derived from strain M-1404. Isolate 314-3 was nonreactive to somatic antisera but had a fingerprint profile that resembled the reference serotype 2 DNA fingerprint profile.

Although isolates among the classic hemorrhagic septicemia *P. multocida* isolates (serotype B:2 or B:2,5) could be further differentiated by DNA fingerprint profiles, a fingerprint profile obtained with the *Hha*I endonuclease was not necessarily unique to a country of origin or host species. For

TABLE 3. Serotypes, origins, and DNA profiles of *P. multocida* capsule serogroup E isolates

Strain	Serotype	DNA profile		Source	Geographic origin
		*Hha*I	*Hpa*II		
P-4096	E:2,5	017	001	Cattle	Zambia
P-4099	E:2,5	017	001	Cattle	Zambia
P-4109	E:2,5	017	002	Cattle	Sudan
P-4112	E:2,5	017	005	Cattle	Sudan
P-4119	E:2,5	017	005	Cattle	Chad
P-4120	E:2,5	017	004	Cattle	Cameroon
P-4121	E:2,5	017	003	Cattle	Senegal
P-4122	E:2,5	017	003	Cattle	Nigeria
P-4123	E:2,5	017	004	Cattle	Mali
P-4367	E:2,5	017	004	NA[a]	Nigeria
P-4097	E:2,5	017	001	Swine	Zambia
P-4098	E:2,5	017	001	Cattle	Zambia
P-4110	E:2,5	017	002	Cattle	Sudan

[a] NA, not available.

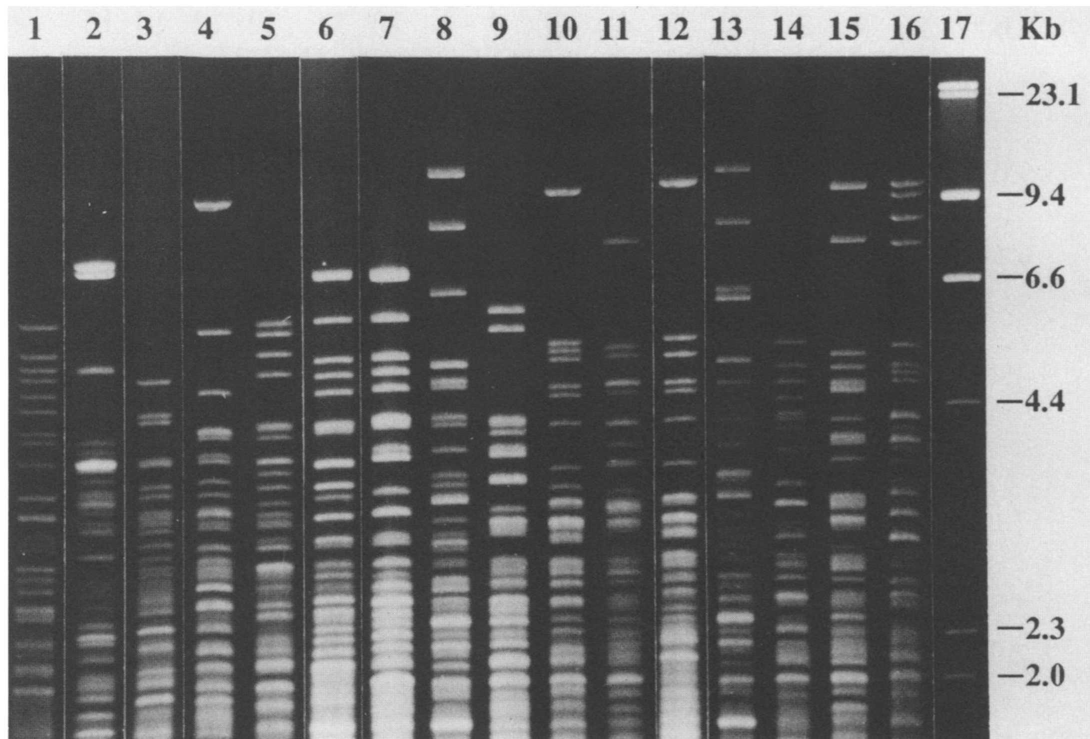

FIG. 1. Agarose gel DNA fingerprint profiles of 16 somatic reference serotype strains. Lanes 1 through 16 contained *Hha*I profiles 001 through 016 of *P. multocida* somatic reference serotype strains 1 through 16. Lane 17 contained DNA from lambda bacteriophage digested with *Hin*dIII.

example, profile 018 was found in swine and cattle from countries as diverse as India, Zimbabwe, and Malaysia.

Sixteen group B isolates that reacted as somatic serotypes (1; 4; 4, sl 12; and 3,4) had DNA fingerprint profiles (Fig. 3) that did not match the profiles of any of the 16 reference somatic serotypes. Neither did these isolates have fingerprint profiles that matched those of any of the classic hemorrhagic septicemia-causing isolates. Seven fingerprint profiles were identified with the *Hha*I endonuclease among these isolates, and they were designated profiles 030 to 036 (Fig. 3). The discrepancy between serotype and fingerprint profile that occurred among the classic hemorrhagic septicemia-causing *P. multocida* isolates also occurred among these 16 isolates.

Comparison of isolates within capsule group E. The 13 capsule group E isolates all reacted as somatic serotype 2,5 (Table 3). One DNA fingerprint profile with *Hha*I was designated 017 and was common to all of the group E isolates (Fig. 2). The 017 profile was not identical to any fingerprint of the 16 somatic reference serotype strains (Fig. 1) or the group B isolates. Although the capsule group E isolates were a homogeneous group upon the basis of serologic typing and *Hha*I profile, they were isolated from different species and countries. The 13 serogroup E isolates were digested with the secondary endonuclease (*Hpa*II), and five unique DNA profiles (Fig. 4) were recognized. All four isolates from Zambia, three of which originated from cattle and one of which came from swine, were identical when the *Hpa*II endonuclease was used. Eight of the remaining nine capsule group E isolates originated from cattle of six countries.

A descriptive identification epithet (DIE) was assigned to each capsule group E isolate for serologic and DNA profile characterization. The combination of the serologic identification and DNA fingerprint profiles of the *Hha*I and *Hpa*II restriction endonucleases in this order is the basis for the DIE code. For example, a serologic type E:2,5 isolate which has fingerprint profiles *Hha*I 017 and *Hpa*II 001 would be described as DIE code E:2,5-*Hha*I 017-*Hpa*II 001. Five DIE codes were recognized from 13 isolates (E:2,5-*Hha*I 017-*Hpa*II 001 through E:2,5-*Hha*I 017-*Hpa*II 005). The sequence of the DIE code indicates the order in which all tests were performed.

DISCUSSION

It is accepted by those working with hemorrhagic septicemia of cattle and buffalo that the disease is caused specifically by *P. multocida* having the group B or E capsule and somatic serotype 2 or 5 antigen. *P. multocida* organisms with the B:2 or B:2,5 formula are occasionally isolated from other species (i.e., swine and deer) in which they produce clinical signs and pathologic lesions that are similar to those seen in cattle and buffalo. Recent evidence indicates that hemorrhagic septicemia in wild ruminants such as deer and elk can also be produced by *P. multocida* with the antigenic formula B:3,4. However, either the incidence of these organisms is rare or they are only rarely reported. Seven DNA fingerprint profiles (*Hha*I 030 to 036) were recognized among 16 serogroup B isolates that reacted to somatic antigens other than serotype 2 or 5. Four different DNA fingerprint profiles were recognized from 10 isolates that had the B:3,4 serologic type. Although the latter are isolated infrequently, a strong immunological relationship exists between them and other serogroup B *P. multocida* isolates. Antibodies against the B

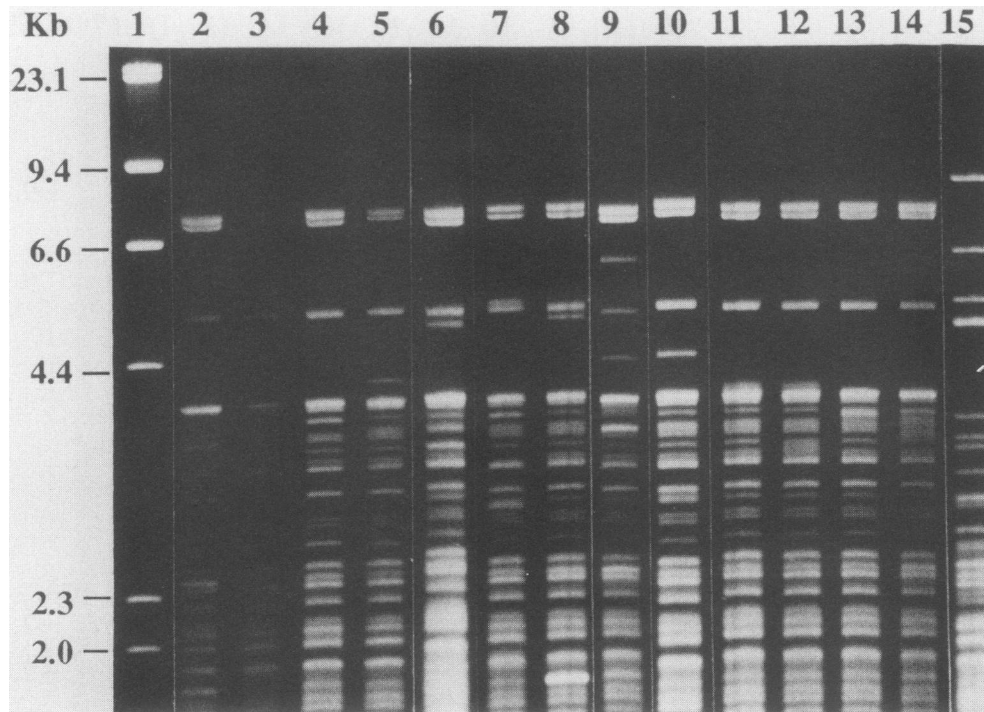

FIG. 2. Agarose gel DNA fingerprint profiles of *P. multocida* serogroup B and E isolates. Lanes: 1, DNA from lambda phage digested with *Hin*dIII; 2 through 14, *Hha*I profiles 002 and 018 through 029 of *P. multocida* serogroup B isolates, respectively; 15, *Hha*I profile 017 common to all 13 serogroup E isolates.

capsule passively protect mice against any serogroup B *P. multocida*, regardless of somatic serotype or host species.

Although serologic typing is useful in the presumptive diagnosis of hemorrhagic septicemia of cattle and buffalo, its use is limited in epidemiologic studies. A potential use of DNA fingerprint profiles in conjunction with serologic typing for epidemiologic surveys of hemorrhagic septicemia-causing *P. multocida* isolates can be seen in the data of Table 2. The largest number of isolates in the study was from swine in India. As shown in Table 2, these strains had a diversity of DNA fingerprint profiles; within the B:2 serotype of the swine isolates from India, seven unique fingerprint profiles were detected.

We examined DNA fingerprinting as a possible method for further differentiation of strains. DNA fingerprinting has been used frequently for characterization of bacterial DNA. The efficiency and timeliness of DNA extraction and precipitation are commonly encountered problems. In this study, 16 DNA samples were extracted, precipitated, and harvested within approximately 2.5 h. Preliminary experiments with selected restriction endonucleases indicated that *Hha*I, which has a 4-bp recognition sequence rich in the bases guanine and cytosine, produced easily distinguished fingerprint profiles with each of the 16 reference somatic serotypes. Each profile was unique. The present study demonstrated that this endonuclease is able to resolve differences among serogroup B isolates of the same somatic type. The largest number of strains in the present study was isolated from swine in India. The finding that these swine strains had a diversity of DNA profiles within one somatic serotype, as evidenced by seven unique fingerprint profiles, indicates the potential of DNA fingerprinting for epidemiologic surveys involving hemorrhagic septicemia-causing *P. multocida* isolates.

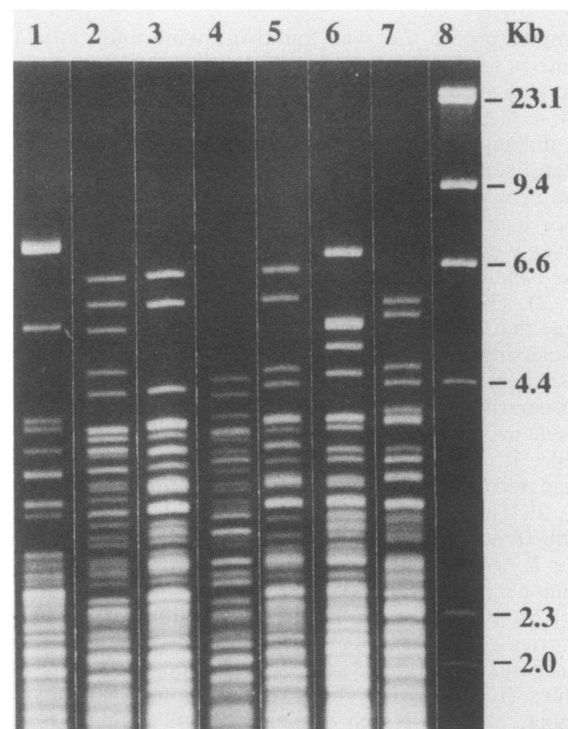

FIG. 3. Agarose gel DNA fingerprint profiles of *P. multocida* serogroup B isolates which did not react with somatic type 2 or 5 antiserum. Lanes: 1 through 7, *Hha*I profiles 031 through 036 and 030, respectively; 8, DNA from lambda phage digested with *Hin*dIII.

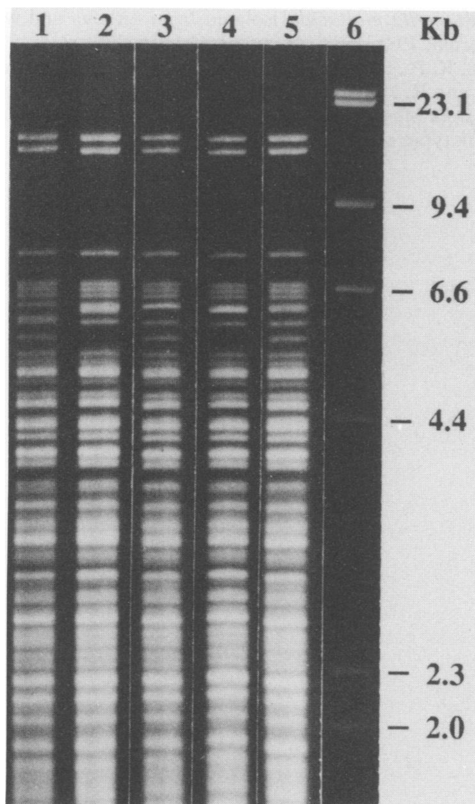

FIG. 4. Agarose gel DNA fingerprint profiles of *P. multocida* serogroup E isolates. Lanes: 1 through 5, *Hpa*II profiles 001 through 005; 6, DNA from lambda phage digested with *Hin*dIII.

Seven unique DNA fingerprint profiles were recognized in 16 serogroup B isolates that were identified as somatic serotype 1; 4; 4, sl 12; or 3,4. The occurrence of separate DNA fingerprint profiles with a single serotype that occurred among the classical hemorrhagic septicemia-causing *P. multocida* isolates also occurred among these 16 isolates. Further differences within all group B isolates might be evident if they were examined with the *Hpa*II endonuclease, as were the serogroup E isolates.

P. multocida isolates of serogroup E are unique in many respects. All previously reported serogroup E isolates have possessed only somatic serotype 2 and 5 antigens. The lipopolysaccharides of these organisms produce identical patterns in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8). Additionally, these organisms have never been isolated outside Africa. The fingerprints of the serogroup E isolates in this study were identical when the *Hha*I endonuclease was used, even though the majority of these isolates originated from cattle in numerous African countries. However, *Hpa*II digestion of our group E isolates produced profiles that could be easily differentiated. Like *Hha*I, *Hpa*II has a four-base recognition sequence but with a different arrangement of guanine and cytosine.

In our study, both restriction endonucleases generated profiles that yield resolution of most fragments between 23.1 and 4.4 kb, yet in some profiles the resolution of fragments near 2.3 kb in size is possible. Use of TBE electrophoresis buffer to resolve DNA fragments larger than 4 kb is common. However, use of Tris-acetate-EDTA buffer has been reported to resolve fragments of 4 kb or less, but recycling of buffer during electrophoresis is necessary.

Resolution of DNA fingerprint profiles by using this procedure with either *Hha*I or *Hpa*II can be accomplished with the naked eye. Occasionally, differences are extremely subtle. The high degree of similarity of serogroup E isolates digested with the *Hpa*II endonuclease can be seen in lanes 3 and 5 of Fig. 4, which appear to be identical at a glance, but after close examination of band seven from the top in both lanes, a difference was observed. In lane 5, this band is actually a doublet.

Analysis of many profiles can accurately be accomplished with computerized restriction fragment length polymorphism equipment. This equipment can be used to create a DNA fingerprint data base and standardize the fragment data from multiple photographic images. For calibration and standardization of restriction fragment length polymorphism equipment, a molecular weight marker must be present on each photographic image. The composition of a computerized fingerprint data base will permit individual isolates to be easily examined. In this study, the *Hha*I endonuclease generated profiles that are easily resolved. The fingerprinting of all *P. multocida* isolates with a routinely used preliminary enzyme such as *Hha*I would make analysis of data easier and less confusing.

Use of serologic identification and *Hha*I and *Hpa*II DNA fingerprint profiles to characterize isolates and generation of a DIE code will provide an orderly means of classification of *P. multocida* isolates that has not been achieved by other methods. Use of serological identification within the DIE code will provide information for evaluation of current capsule and somatic typing methods; however, the absence of serologic data would not affect the fingerprint profile data of the DIE code.

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