Serotypes and DNA Fingerprint Profiles of Pasteurella multocida Isolated from Raptors

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SUMMARY. *Pasteurella multocida* isolates from 21 raptors were examined by DNA fingerprint profile and serotyping methods. Isolates were obtained from noncaptive birds of prey found in 11 states from November 28, 1979, through February 10, 1993. Nine isolates were from bald eagles, and the remaining isolates were from hawks, falcons, and owls. Seven isolates were members of capsule group A, and 14 were nonencapsulated. One isolate was identified as somatic type 3, and another was type 3,4,7; both had unique *HpaII* DNA fingerprint profiles. Nineteen isolates expressed somatic type 1 antigen; *HpaII* profiles of all type 1 isolates were identical to each other and to the *HpaII* profile of the reference somatic type 1, strain X-73. The 19 type 1 isolates were differentiated by sequential digestion of DNA with *HpaII*; four *HpaII* fingerprint profiles were obtained. The *HpaII* profile of one isolate was identical to the *HpaII* profile of strain X-73. Incidence of *P. multocida* somatic type 1 in raptors suggests that this type may be prevalent in other wildlife or wildlife environments.

RESUMEN. Serotipos y perfiles dactilares del ADN de cepas de *Pasteurella multocida* aislada de aves de rapina.

Por medio de la serotipificación y por el examen de los perfiles del ADN, se examinaron 21 cepas de *Pasteurella multocida* aisladas de aves de rapina. Las muestras fueron obtenidas de aves de rapina de vida libre encontradas en 11 estados desde Noviembre 28 de 1979 hasta Febrero 10 de 1993. Nueve cepas eran de águilas calvas, y las cepas restantes se obtuvieron de halcones, buitres y buhos. Siete cepas pertenecían al grupo capsular A y 14 no tenían cápsula. Una cepa se identificó como del tipo somático 3, otra fue del tipo 3,4,7; ambas tenían perfiles incomparables de ADN *HpaII*. Diez y nueve cepas expresaron el antígeno somático de tipo 1, los perfiles *HpaII* de todas las cepas del tipo 1 fueron idénticos entre sí lo mismo que al perfil *HpaII* de la cepa de referencia X-73 para el tipo somático 1. Las cepas tipo 1 fueron diferenciadas mediante la digestión secuencial del ADN con *HpaII*, obteniéndose cuatro perfiles diferentes. El perfil de una cepa fue idéntico al perfil *HpaII* de la cepa X-73. La incidencia de *P. multocida* del tipo 1 somático en aves de rapina sugiere que este tipo puede ser prevalente en otros animales salvajes o en otros ambientes selváticos.

Key words: *Pasteurella multocida*, DNA fingerprinting, raptors, serotyping, fowl cholera

Predation is an important component of nature and a means of existence for raptors. Some use piracy to secure food, but the easiest catch is carrion. Diseased animals are also prey, and those that die of disease are one form of carrion consumed by raptors. Consequently, raptors can be victims of disease due to inherent food-gathering habits. Host susceptibility and environ-
raptors to *P. multocida* are not well documented. Serotypic characterization of *P. multocida* from domestic and wild animals has often been used (9,12,17,18). Precise characterization of *P. multocida* from wildlife and domestic livestock by fingerprinting is crucial for establishing relatedness.

Various wildlife species have been identified as potential reservoirs of fowl cholera for domestic poultry (6); in that study, DNA fingerprint relationships were not established among serotype A:1 isolates of domestic or wild animal origin, whereas profile relationships were established among serotype A:3 or A:3,4 isolates originating from small rodents, sparrows, and turkeys.

Serotypic identification of *P. multocida* by capsular and somatic typing distinguishes antigenic or phenotypic characteristics of isolates. Antigenic expression identified by serotyping provides important information, although ambiguous or misleading characterization often results. Numerous antigenically distinct *P. multocida* serotypes have been documented (17). Other methods for characterization of *P. multocida* (14,22) have also been reported.

DNA fingerprint analysis of *P. multocida* isolated from domestic poultry has been reported (5). DNA fingerprint analysis of isolates has been used with serotypic characterization for creating a *P. multocida* identification scheme (23). Results from these procedures are expressed as a descriptive identification epithet (DIE) code. The DIE code consists of serological identification and fingerprint profile designation with one or more endonucleases. For example, a serologic type A:1 isolate that has fingerprint profiles *HbaI* 001 and *HpaII* 004 would be described as DIE code A:1-*HbaI* 001/*HpaII* 004. The characteristics of an isolate are described phenotypically and genotypically.

The purpose of this study was to determine fingerprint profiles and serotypes of *P. multocida* isolated from raptors and to compare these with each other and with 16 somatic reference strains.

**MATERIALS AND METHODS**

Twenty-one *P. multocida* isolates from raptors were examined; Table 1 shows isolates by host species, date of isolation, and state of origin. All isolates were recovered from tissue of diseased birds of prey. Twenty raptors had been submitted to the National Wildlife Health Center, Madison, Wis., for diagnostic purposes. Initial propagation of *P. multocida* was on blood agar base plates containing 5% sheep blood. One isolate from a prairie falcon had been recovered by a veterinary practitioner and sent to the National Wildlife Health Center for confirmation. All isolates were biochemically characterized by the API-20E (Analytab Products, Plainview, N.Y.) system. Isolates were frozen (−70°C) in a glycerin-based cryoprotectant for long-term storage.

*Pasteurella multocida* for DNA fingerprinting was propagated on Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 1.25% tryptose broth (23). Isolates were serotyped by capsule (19) and somatic (10) typing methods; passive hemagglutination and gel diffusion precipitin tests were used, respectively. Serotyping results reported in this study are those of the National Veterinary Services Laboratories.

DNA isolation and digestion with *HbaI* or *HpaII* (Life Technologies, Inc., Gaithersburg, Md.), electrophoresis, and photographic techniques have been described (23). DNA from each isolate was first digested with *HbaI*. DNA from isolates that have identical *HbaI* fingerprint profiles was sequentially digested with *HpaII*. Molecular weight of fragments was determined by using DNA from lambda bacteriophage digested with *HindIII* as a gel marker.

**RESULTS**

**Serotyping.** Six *P. multocida* serotypes (--1; -1,7; -1,3,7; A:1; A:1,7; and A:1,3,7) were identified from 19 of 21 raptors (Table 1). These isolates expressed somatic type 1 antigen and are collectively referred to as somatic type 1. The remaining two isolates were identified as serotypes --3 and A:3,4,7.

**DNA fingerprint profiles of isolates with *HbaI***. Profile designations of 16 *P. multocida* somatic reference strains have been documented (23). Nineteen *P. multocida* isolates from raptors had *HbaI* fingerprint profiles identical to the *HbaI* profile of somatic reference type 1, strain X-73 (Fig. 1; profile *HbaI* 001). The fingerprint profiles of two raptor isolates (7535-001 and 8512-001, Fig. 2) failed to match the *HbaI* profiles of 16 somatic reference strains. For organization of a DIE code scheme, these profiles were temporarily designated (TD) *HbaI* TD 064 and *HbaI* TD 065, respectively.

**DNA fingerprint profiles of 19 somatic type 1 isolates with *HpaII***. Four *HpaII* profiles were identified among the 19 somatic type 1 isolates (Fig. 3). Differences among lanes 2, 3, and 4 are found in the 6.6-kilobase region;
Table 1. Serotypes, origins, and DNA fingerprint profiles of *Pasteurella multocida* isolates from raptors from the United States.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Raptor</th>
<th>Isolation date</th>
<th>State</th>
<th>Serotype*</th>
<th><em>HpaiI</em> profile designation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>6698-1</td>
<td>Bald eagle</td>
<td>12-12-86</td>
<td>Maine</td>
<td>-1</td>
<td>001</td>
</tr>
<tr>
<td>1649-16156</td>
<td>Bald eagle</td>
<td>11-28-79</td>
<td>Minn.</td>
<td>-1</td>
<td>002</td>
</tr>
<tr>
<td>2815-29462</td>
<td>Bald eagle</td>
<td>05-28-82</td>
<td>Ore.</td>
<td>-1,3,7</td>
<td>003</td>
</tr>
<tr>
<td>1858-19146</td>
<td>Bald eagle</td>
<td>04-01-82</td>
<td>Calif.</td>
<td>-1</td>
<td>003</td>
</tr>
<tr>
<td>6749-001</td>
<td>Bald eagle</td>
<td>01-22-87</td>
<td>Wis.</td>
<td>A:1</td>
<td>003</td>
</tr>
<tr>
<td>5180-001</td>
<td>Bald eagle</td>
<td>02-20-85</td>
<td>Calif.</td>
<td>-1</td>
<td>004</td>
</tr>
<tr>
<td>7618-001</td>
<td>Bald eagle</td>
<td>12-23-87</td>
<td>Missouri</td>
<td>A:1</td>
<td>004</td>
</tr>
<tr>
<td>10031-1</td>
<td>Bald eagle</td>
<td>03-18-91</td>
<td>Ill.</td>
<td>-1</td>
<td>004</td>
</tr>
<tr>
<td>10494-1</td>
<td>Bald eagle</td>
<td>01-09-92</td>
<td>Neb.</td>
<td>A:1</td>
<td>004</td>
</tr>
<tr>
<td>2746-28980</td>
<td>Marsh hawk</td>
<td>02-04-82</td>
<td>Colo.</td>
<td>-1</td>
<td>003</td>
</tr>
<tr>
<td>2811-29446</td>
<td>Marsh hawk</td>
<td>03-25-82</td>
<td>Calif.</td>
<td>-1</td>
<td>004</td>
</tr>
<tr>
<td>5461-002</td>
<td>Red-shouldered hawk</td>
<td>04-30-85</td>
<td>Calif.</td>
<td>A:1,7</td>
<td>003</td>
</tr>
<tr>
<td>11264-002</td>
<td>Red-tailed hawk</td>
<td>01-27-93</td>
<td>Calif.</td>
<td>-1,7</td>
<td>004</td>
</tr>
<tr>
<td>11266-001</td>
<td>Peregrine falcon</td>
<td>01-25-93</td>
<td>Calif.</td>
<td>-1,3,7</td>
<td>004</td>
</tr>
<tr>
<td>6607-1</td>
<td>Prairie falcon</td>
<td>10-30-86</td>
<td>Neb.</td>
<td>-1</td>
<td>004</td>
</tr>
<tr>
<td>1675-17028</td>
<td>Short-eared owl</td>
<td>12-07-79</td>
<td>Calif.</td>
<td>-1</td>
<td>003</td>
</tr>
<tr>
<td>5461-001</td>
<td>Burrowing owl</td>
<td>11-19-85</td>
<td>Calif.</td>
<td>A:1,3,7</td>
<td>003</td>
</tr>
<tr>
<td>10464-1</td>
<td>Barn owl</td>
<td>11-29-91</td>
<td>Idaho</td>
<td>-1,7</td>
<td>004</td>
</tr>
<tr>
<td>11324-006</td>
<td>Long-eared owl</td>
<td>02-10-93</td>
<td>Ore.</td>
<td>A:1</td>
<td>004</td>
</tr>
<tr>
<td>7535-001</td>
<td>Hawaiian owl</td>
<td>11-08-87</td>
<td>Hawaii</td>
<td>-3</td>
<td>NA*</td>
</tr>
<tr>
<td>8512-001</td>
<td>Spotted owl</td>
<td>02-02-89</td>
<td>Calif.</td>
<td>A:3,4,7</td>
<td>NA*</td>
</tr>
</tbody>
</table>

*Letter = capsule group; number = somatic type; – denotes nonencapsulated strain.
*Designated *HpaiI* profile number.
*NA = not applicable; isolates were not identified as somatic type 1.

and lane 5 has a band at the 9.4-kilobase region (Fig. 3). These were designated as *HpaiI* profiles 001 through 004 and corresponded to DII codes A:1–*HbaI* 001–*HpaiI* 001 through A:1–*HbaI* 001–*HpaiI* 004 (Table 1). Sixteen of 19 somatic type 1 isolates were from raptors found in states west of the Mississippi River. Isolates 10031-1, 6749-001, and 6698-1 originated from birds found in Illinois, Wisconsin, and Maine, respectively. Only isolate 6698-1 had a *HpaiI* profile identical to reference somatic type 1, strain X-73, designated A:1–*HbaI* 001–*HpaiI* 001. Somatic reference type 1, strain X-73, originated from a chicken in Maryland. Although few *P. multocida* isolates from raptors were evaluated in this study, it is noteworthy that 6698-1 and X-73 had identical *HpaiI* profiles and that they originated from the East Coast.

**DISCUSSION**

In our study, the high incidence (90.5%) of somatic type 1 *P. multocida* in raptors could result from several factors. The incidence of somatic type 1 *P. multocida* is higher in waterfowl than in domestic avian species (1,4,24,25). This suggests that wild waterfowl could be a reservoir of A:1 *HbaI* 001 for raptors. Eagles often prey on wild waterfowl and have been associated with fowl cholera epizootics. Although this explains a potential source of somatic type 1 *P. multocida* for eagles, it provides no account of a reservoir for other raptors such as nocturnal owls. Using only serotyping methods, isolates can appear phenotypically similar or identical; thus, wildlife could be considered a reservoir of fowl cholera for domestic poultry. Somatic type 1 *P. multocida* is often found in mice and rats, suggesting that serotype A:1 may be well adapted to the environment. Characterization of *P. multocida* from mice and rats with *HbaI* may identify these species as a potential reservoir of disease.

The incidence of somatic type 1 *P. multocida* isolated from domestic poultry varies by report (1,12,17,24); either *P. multocida* somatic type 3 or 3,4 was the most common type identified. Relative incidence of somatic type 1 *P. multo-
P. multocida isolated from domestic poultry is affected by use of somatic type 3,4 attenuated vaccines for control of fowl cholera in domestic poultry. Other factors affecting isolation of somatic types in those reports include the number of isolates examined from a geographic region, density of poultry in a region, size of the region studied, and duration of the study.

By using DNA fingerprinting, it is possible to establish relationships or differences between isolates of P. multocida of similar or different somatic type. DNA fingerprint studies of P. multocida originating from domestic poultry often identify numerous profiles attributable to a somatic type. The HbaI profiles of a few somatic type 1 P. multocida isolates from domestic poultry have been reported (24); those HbaI profiles had heterogeneous banding patterns and did not match the profile of strain X-73. However, heterogeneity among P. multocida DNA fingerprint profiles with HbaI was not observed among 19 raptor somatic type 1 isolates. This suggests that domestic poultry are seldom reservoirs of somatic type 1 P. multocida for raptors. Rats, mice, opossum, sparrows or other small birds, and cats are often considered potential reservoirs of P. multocida for domestic poultry. Frequently, however, the reservoir or source of fowl cholera affecting domestic poultry and wildlife is unknown.

In contrast, DNA fingerprint heterogeneity using SmaI was recognized (6) among somatic type 1 P. multocida of wildlife origin. Unfortunately, we cannot compare reports involving different endonucleases. We suggest that HbaI should be used as preliminary endonuclease for P. multocida DNA fingerprinting, because profiles with well-resolved fragments are observed.
wildlife. Establishment of precise isolate characterization allows evaluation of reservoir status, wildlife management, and disease control practices.

**REFERENCES**


