Molecular Identification and Epidemiological Tracing of Pasteurella multocida Meningitis in a Baby

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We report a case of Pasteurella multocida meningitis in a 1-month-old baby exposed to close contact with two dogs and a cat but without any known history of injury by these animals. 16S rRNA gene sequencing of the isolate from the baby allowed identification at the subspecies level and pointed to the cat as a possible source of infection. Molecular typing of Pasteurella isolates from the animals, from the baby, and from unrelated animals clearly confirmed that the cat harbored the same P. multocida subsp. septica strain on its tonsils as the one isolated from the cerebrospinal fluid of the baby. This case stresses the necessity of informing susceptible hosts at risk of contracting zoonotic agents about some basic hygiene rules when keeping pets. In addition, this study illustrates the usefulness of molecular methods for identification and epidemiological tracing of Pasteurella isolates.

A previously healthy 1-month-old baby from a rural area of Switzerland was admitted to the pediatric ward of the local hospital in the winter of 1998 to 1999. The baby presented with an irritable state, a temperature of 39°C, and signs of slightly increased intracranial pressure. A lumbar puncture was performed, and the cerebrospinal fluid (CSF) was positive for P. multocida subsp. septica strain on its tonsils as the one isolated from the cerebrospinal fluid of the baby. This case stresses the necessity of informing susceptible hosts at risk of contracting zoonotic agents about some basic hygiene rules when keeping pets. In addition, this study illustrates the usefulness of molecular methods for identification and epidemiological tracing of Pasteurella isolates.
ter profile was identical to the profile obtained with an isolate from one of the dogs which had also been in contact with the baby. Another isolate from the second dog which had contacts with the baby also had a biochemical profile compatible with an unidentifiable Pasteurella species (data not shown). 16S rRNA gene sequencing did not allow further identification of the latter three isolates to the species level but confirmed their affiliation with the Haemophilus-Pasteurella-Actinobacillus cluster and clearly distinguished them from the baby’s isolate (6% or more sequence divergence compared to the P. multocida subsp. septica isolate from the baby and to the Pasteurella multocida subsp. septica cluster in general).

The P. multocida subsp. septica isolates were typed by ribotyping and macrorestriction analysis. Ribotyping was performed as described elsewhere (8). Briefly, DNA was extracted using guanidium thiocyanate (20) and digested with the restriction enzymes HindIII and EcoRI following the manufacturer’s (Roche Diagnostics, Rotkreuz, Switzerland) instructions. The resulting fragments were separated by agarose gel electrophoresis and transferred by vacuum blotting on positively charged nylon membranes (Roche Diagnostics). A digoxigenin-labeled probe specific for 16S rRNA genes was prepared by PCR and used for hybridizations, and the hybridization patterns were revealed using the digoxigenin luminescence kit (Roche Diagnostics). For macrorestriction analysis, bacteria were grown on sheep blood agar plates and resuspended in TE (10 mM Tris, 1 mM EDTA, pH 8.0) to a final optical density at 600 nm of 4.0. The suspensions were mixed with an equal volume of melted 1.2% SeaKem Gold agarose (FMC, Rockland, Maine), and 1-mm-thick plugs were prepared. The plugs were incubated overnight at 50°C in 0.5 M EDTA–1% lauroyl-sarcosine containing 2 mg of proteinase K per ml and washed five times for 45 min each time in TE. The DNA was subsequently digested for 4 h with 30 U of SalI or SmalI under conditions described by the manufacturer (Roche Diagnostics). The resulting DNA fragments were separated with a CHEF III electrophoresis system (Bio-Rad, Hercules, Calif.) in 1% SeaKem Gold agarose gels (FMC) for 16 h in 0.5 × Tris-borate-EDTA at 14°C by applying an electric field of 6 V/cm with an angle of 120°C and a linear ramping ranging of 1.5 to 17 s. Gels were stained in 1 mg of ethidium bromide per ml and destained in water before photography under UV light.

Results of ribotyping and macrorestriction analysis are summarized in Table 1. Only three very similar ribotypes could be distinguished when using the restriction enzyme HindIII, with nine isolates belonging to a single type (Table 1). These three ribotypes were clearly different from those recently described for P. multocida subsp. multocida (9), thus confirming their identification as P. multocida subsp. septica. EcoRI was more discriminatory (Table 1), but the profiles obtained with this enzyme remained relatively similar and were difficult to read because of the presence of variable bands in the high-molecular-weight range (data not shown). The isolates from the baby and from the cat had identical ribotypes with both restriction enzymes. Macrogenstriction profiles were easy to read and allowed us to distinguish all of the P. multocida subsp. septica isolates from one another, except those from the baby and the contact cat, which remained identical (Fig. 1).

Cattle, pigs, and poultry have been incriminated in the past as the major sources of human infections with zoonotic agents. However, humans have very frequent and close contacts with pets, an often forgotten source of zoonoses. In the particular case of Pasteurella infections, animal bites and scratches are the major sources of infection for humans (11). P. multocida subsp. multocida represents the majority of Pasteurella isolates from these cases, followed by a smaller proportion of P. multocida subsp. septica. Pasteurella strains have been suggested by others as a possible infection route for those patients without injury (7, 12) and is likely to have occurred in the present case. A particular tropism of P. multocida subsp. septica for the central nervous system has been suggested by others (4), which fits our particular case.

Table 1. Ribotypes and macrogenstriction profiles obtained with 11 P. multocida subsp. septica isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Ribotyping analysis</th>
<th>Macrogenstriction analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HindIII</td>
<td>EcoRI</td>
</tr>
<tr>
<td>D25/99</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D129/99</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>D138/99</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>D176/99</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>D496/99</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>D514/99</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>D755/99</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>D777a/99</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>D777b/99</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>D590/99</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>D708/99</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

* The numbers in columns 2 to 5 represent random numbering of the profiles obtained by ribotyping and macrogenstriction analysis.

1. P. multocida subsp. septica from the baby.
2. P. multocida subsp. septica isolates from eight epidemiologically unrelated cats.
3. One of nine P. multocida subsp. septica isolates from eight epidemiologically unrelated cats.
4. The numbers in columns 2 to 5 represent random numbering of the profiles obtained by ribotyping and macrogenstriction analysis.

canis and P. multocida subsp. septica (12). P. multocida subsp. multocida has been previously associated with dogs, and P. multocida subsp. septica has been associated with cats (4). Analysis of our sequence database also confirms the strong association of clinical isolates of the P. multocida subsp. septica 16S rRNA gene cluster with cats (data not shown), including isolates with atypical biochemical profiles (one ornithine decarboxylase-negative isolate and several isolates with variable reactions for trehalose and sorbitol). Especially the subspecies may be difficult to identify using conventional phenotypic testing. In contrast, the identification of P. multocida subsp. septica by 16S rRNA gene sequencing at the subspecies level was unequivocal for the CSF isolate of the baby and therefore pointed to the cat as the origin of the infection. This hypothesis was also supported by previous reports of transmission of P. multocida subsp. septica between cats and babies (2, 18). As in the present case, a significant proportion of P. multocida infections in humans are associated with animal exposure without any injury (3, 10, 13, 16, 19). Moreover, some systemic infections can even not be associated with any animal exposure at all (7, 16). In such cases, only the comparison of environmental and clinical isolates with discriminatory and reproducible typing methods is able to demonstrate any link between a potential source of infection and a particular clinical case. Molecular typing methods like restriction enzyme analysis (16, 23), random amplification of polymorphic DNA (20), ribotyping (2, 9), and macrogenstriction analysis (6, 14, 22) have been used for typing of members of the genus Pasteurella and have been shown to be good tools for epidemiological tracing. In the present case, the molecular analysis of the isolates demonstrated the identity of the P. multocida subsp. septica strains from the cat and the baby. The analysis of additional, unrelated P. multocida subsp. septica isolates demonstrated the high discriminatory power of ribotyping and particularly of macrogenstriction analysis for this Pasteurella subspecies and confirmed that the identity of the profiles for the cat and human isolates was not fortuitous. Inhalation or licking and oral transmission has been suggested by others as a possible infection route for those patients without injury (7, 12) and is likely to have occurred in the present case. A particular tropism of P. multocida subsp. septica for the central nervous system has been suggested by others (4), which fits our particular case.

To our knowledge, this is the first report of a P. multocida...
subsp. septica meningitis in which the organism was transmitted without any injury from a cat to a baby to be clearly documented by genotypic and molecular epidemiological methods. The baby had been massively exposed to the dogs by ingestion of Salmonella L-amino acid and by direct contact with the dog. Finally, the present case stresses the danger of casual contact between humans and pets. In view of the very frequent contacts between pets and humans (10) and the low number of reported Pasteurella multocida infections, the risk of transmission of this zoonotic agent from domestic animals to humans is probably very low. However, some basic rules of hygiene should be respected when immunocompromised patients, small children, and the elderly come into contact with pets (7, 16). Predisposed persons may have to be made aware of these precautions. Since the limited phenotypic identification tests applicable in a routine diagnostic laboratory would have misidentified several of the P. multocida subsp. septica isolates used in this study, the present work exemplifies the need for molecular identification methods for taxa such as members of the family Pasteurellaceae. Finally, our results also clearly illustrate the potentials of pulsed-field gel electrophoresis for epidemiological tracing of the occurrence of P. multocida infections in humans.

REFERENCES