Cat-Contaminated Environmental Substances Lead to \textit{Yersinia pseudotuberculosis} Infection in Children

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A 1-year-old boy was infected with \textit{Yersinia pseudotuberculosis} serotypes 1b and 3, and his 3-year-old brother was infected with \textit{Y. pseudotuberculosis} serotype 1b; both had drunk water from puddles in a garden of their housing district of Miyoshi City, Hiroshima Prefecture, Japan. The \textit{Y. pseudotuberculosis} serotype 1b and 3 strains isolated from soil from the dried-up puddles and sand and feces from the sandbox proved to be from a stray cat. The restriction endonuclease patterns of the plasmid in each strain of \textit{Y. pseudotuberculosis} serotypes 1b and 3 were identical. These data provide evidence for the transmission of \textit{Y. pseudotuberculosis} through water, sand, and soil contaminated by feces from cats infected with this species.

\textit{Yersinia pseudotuberculosis} causes sporadic and epidemic infections in humans and is widely distributed among domestic pets (5, 6, 13, 17, 18), farm animals (17), and wild animals (2, 3, 17). Fukushima et al. (4) reported that \textit{Y. pseudotuberculosis} infection may be related to contact with environmental substances contaminated with \textit{Y. pseudotuberculosis} during the cold months. Pets such as dogs and cats may become a source of infection with \textit{Y. pseudotuberculosis} since they are infected with this organism during the cold months and excrete up to $10^8$ cells per g of feces (5, 6).

There are reports in which human \textit{Y. pseudotuberculosis} infections followed infection of a family pet such as a cat or dog (12, 14). Paul and Weltmann (14) reported such findings in a laborer who died of \textit{Y. pseudotuberculosis} septicemia 8 days after working in a garden where the soil had been fouled by the excreta of a cat with diarrhea and also that the same strain of \textit{Y. pseudotuberculosis} was isolated from the liver and spleen of the cat. Macaulay et al. (12) found agglutinins against the infecting organism in the serum of a dog which belonged to a man who had died of yersinial septicemia and which had bitten the patient some days before the onset of his illness. However, the mode of transmission of the organism to humans in such cases is not well understood.

We have now obtained evidence for the transmission of \textit{Y. pseudotuberculosis} through water contaminated by feces from a \textit{Y. pseudotuberculosis}-infected cat.

\textbf{Materials and Methods}

\textbf{Culture methods.} Five stool specimens were collected from two symptomatic patients (1- and 3-year-old brothers) and their healthy parents and 5-year-old brother. The following environmental samples were examined bacteriologically (Table 1). On 25 to 30 March, one sample of the city drinking water, the feces of one parakeet, one sample of the soil from the dried-up puddles (from which the water had been drunk by the 1- and 3-year-old brothers) in the garden, and one sample of sand from the sandbox were collected. On 9 and 10 April, 17 samples of soil in the garden and six stools from a stray cat in the sandbox were collected. The six stools were rod shaped, dry, and hard. Three rats (\textit{Apodemus speciosus}) were trapped in a bush about 500 m from the residence of the boys. Stool specimens from each person were tested for a wide range of enteric bacterial pathogens. Salmonella-shigella agar, deoxycholate-hydrogen sulfide-lactose agar, vibrio agar, and bromthymol blue-Teepol agar (all from Nissui Pharmaceutical Co., Tokyo, Japan) were streaked with the stool specimens and incubated at 37°C aerobically for 24 h. Campylobacter-selective medium (Skirrow) was streaked with the stool specimens and incubated at 42°C microaerobically for 48 h. The numbers of \textit{Y. pseudotuberculosis} cells in stool specimens and environmental specimens were determined (as CFU) at 32°C by aerobic incubation for 48 h (3, 6) on a plate of Irgasan-Novobiocin agar (3). Thirty colonies on the plate (on which 30 to 300 colonies having the characteristics of \textit{Y. pseudotuberculosis} grew) were examined biochemically and serologically. One gram each of stool specimens from each person and the feces from the parakeet, cat, and rodents was suspended in 10 ml of 0.067 M phosphate-buffered saline. One gram each of soil and sand was suspended in 10 ml of peptone-mannitol-phosphate buffer solution (7). These suspensions were incubated at 4°C for 3 to 5 weeks. The suspensions were then subcultured on Irgasan-Novobiocin agar with KOH after enrichment (1). \textit{Y. pseudotuberculosis} was identified by the method of Bercovier et al. (2), and serological grouping of \textit{Y. pseudotuberculosis} was performed with O antisera prepared against O groups 1 to 8 of \textit{Y. pseudotuberculosis} (15, 16).

\textbf{Assay of virulence-associated properties.} All isolates identified as \textit{Y. pseudotuberculosis} were examined for calcium dependency at 37°C by growth on magnesium oxalate agar (8), autoagglutination at 37°C (11) with tryptic soy broth, and pyrazinamidase activity (9). Plasmid DNA was detected by the method of Kaneko and Maruyama (10). The restriction endonuclease patterns of the \textit{Y. pseudotuberculosis} serotype 1b and 3 strains were compared. Plasmid DNA isolated from each strain was digested with restriction endonucleases \textit{Bam}HI, \textit{Eco}RI, and \textit{Hind}III and subjected to 0.7% agarose gel electrophoresis.

\textbf{Serologic methods.} Serum specimens were collected sev-
eral times from patients and twice from parents between 26 March and 21 April. The sera were titrated for O agglutinins against the _Y. pseudotuberculosis _serotype 1b strains isolated from each patient and against the _Y. pseudotuberculosis _serotype 3 strain isolated from the 1-year-old boy. The antigens were prepared from isolated strains that had been cultivated at 25°C for 48 h on tryptic soy agar. The harvested cultures were suspended in saline, washed, and heated at 100°C for 1 h to be used for tube agglutination.

**RESULTS**

**Patients.** A 1-year-old boy (patient 1) and his older brother (patient 2, a 3-year-old boy) drank water from puddles in a garden of their housing unit in Miyoshi City, Hiroshima Prefecture, Japan, on 2 March 1988. Patient 1 became ill on day 12 after drinking this water (14 March). Patient 2 became ill on day 16 after drinking this water (18 March).

**Patient 1 case history.** Patient 1 became ill with a slight fever (37°C) and rash and was admitted to a nearby clinic of a general practitioner on 14 March. The rash on the foot continued for 3 days, and the slight fever, poor appetite, constipation, and sick feeling persisted. On 21 March the patient was admitted to the Department of Pediatrics, Shiman Medical University, Shiman Prefecture, Japan. On admission, he was somnolent and had a fever (39.4°C) and pharyngitis. For several days thereafter, fever, diarrhea, abdominal pain, strawberry tongue, cervical lymphadenopathy, hepatomegaly, and acute renal failure persisted. On 30 March desquamation occurred. The patient recovered on conservative treatment and was discharged on 15 April.

Laboratory data on 21 March included the following: hemoglobin, 10.1 g/dl; hematocrit, 30.1%; leukocyte count, 34,000/mm³; blood urea nitrogen, 33 mg/dl; Na, 123 meq/liter; K, 4.6 meq/liter; and C-reactive protein, 17.03 mg/dl.

_Y. pseudotuberculosis _serotype 1b and 3 strains were isolated from his feces at 7.7 × 10⁴ cells per g and 2.3 × 10⁴ cells per g, respectively, on 21 March. The agglutinin titers of sera against the serotype 1b and 3 strains were elevated to 160 on day 26 of infection (Table 2).

**Patient 2 case history.** Patient 2 became ill with fever (39°C) on 18 March and had a rash on his feet on 20 March. He was admitted to the same hospital on 25 March. On admission, fever (39.5°C), diarrhea, vomiting, and pharyngitis were present. For several days, fever, diarrhea, abdominal pain, hepatomegaly, and mild acute renal failure persisted. On 14 April desquamation occurred. The patient recovered on conservative treatment and was discharged on 15 April. Laboratory data on 25 March included the following: hemoglobin, 12.3 g/dl; hematocrit, 37.8%; leukocyte count, 16,800/mm³; blood urea nitrogen, 9 mg/dl; Na, 130 meq/liter; K, 4.1 meq/liter; and C-reactive protein, 1.79 mg/dl.

_Y. pseudotuberculosis _serotype 1b was isolated from his feces at 2.5 × 10⁷ cells per g on 25 March. The agglutinin titers of sera against the serotype 1b strain were elevated to 640 on day 36 of infection. The agglutinin titers of sera against the serotype 3 strain were <40 (Table 2).

**Household and environmental specimens.** Table 1 shows the results of epidemiological examinations. _Y. pseudotuberculosis _serotype 1b and 3 strains were each simultaneously isolated from one sample of soil from the dried-up water puddles and one sample of sand in the sandbox collected on 30 March and from one sample of feces from a stray cat collected on 9 April. A _Y. pseudotuberculosis _serotype 1b strain was isolated from one other sample of feces from a stray cat collected on 9 April. _Y. pseudotuberculosis _serotype 1b and 3 strains were present at 4.1 × 10⁷ and 7.0 × 10² cells per g, respectively, in one sample of soil, collected on 30 March. Other strains of _Y. pseudotuberculosis _from sand and feces of a stray cat and rodents were isolated by enrichment cultures. _Y. pseudotuberculosis _serotype 4a and 5a strains were simultaneously isolated from the feces of one

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**TABLE 1. Isolation of _Y. pseudotuberculosis_**

<table>
<thead>
<tr>
<th>Date (1988)</th>
<th>Samples</th>
<th>No. of samples</th>
<th>No. of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 March</td>
<td>Patient 1 (feces)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>25 March</td>
<td>Patient 1 (feces)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Father (feces)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mother (feces)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Old brother (feces)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>City drinking water</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>26 March</td>
<td>Parakeet (feces)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>30 March</td>
<td>Soil from puddles</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sand in sandbox</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9 April</td>
<td>Soil in garden</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Stray cat (feces)</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Rodents (feces)</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

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**TABLE 2. Agglutinin titers against _Y. pseudotuberculosis _serotypes 1b and 3**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Subject</th>
<th>March</th>
<th>April</th>
</tr>
</thead>
<tbody>
<tr>
<td>1b</td>
<td>Patient 1</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Patient 2</td>
<td>&lt;40</td>
<td>&lt;40</td>
</tr>
<tr>
<td></td>
<td>Father</td>
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<td>&lt;40</td>
</tr>
<tr>
<td></td>
<td>Mother</td>
<td>&lt;40</td>
<td>&lt;40</td>
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<tr>
<td>3</td>
<td>Patient 1</td>
<td>&lt;40</td>
<td>40</td>
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<tr>
<td></td>
<td>Patient 2</td>
<td>&lt;40</td>
<td>&lt;40</td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td>&lt;40</td>
<td>&lt;40</td>
</tr>
<tr>
<td></td>
<td>Mother</td>
<td>&lt;40</td>
<td>&lt;40</td>
</tr>
</tbody>
</table>

a Patients drank water from puddles.

b Patient 2 became ill. _Y. pseudotuberculosis _serotypes 1b and 3 were isolated from his feces on 21 March.

c Patient 2 became ill. _Y. pseudotuberculosis _serotype 1b was isolated from his feces on 25 March.
drinking water on days 22 and 23 after this episode. These results strongly suggest that the water in the puddles was the source of the infection.

On day 37 after this episode, the serotype 1b and 3 strains were simultaneously isolated from the feces of a stray cat. However, serotype 1b and 3 strains were not isolated from 17 environmental samples, although serotype 1b and 3 strains were found at a concentration of $10^3$ to $10^4$ cells per g of soil collected on day 27 after this episode. These results show that *Y. pseudotuberculosis* cannot survive for long in environmental substances such as soil in a garden or sand in a sandbox. Our observations strongly suggest that *Y. pseudotuberculosis* in the soil and sand was not the source of infection for the stray cat but rather were contaminated by the feces of the stray cat infected with this species. When animals are infected with multiple serotypes of *Y. pseudotuberculosis*, they can become a source of infection of humans with multiple serotypes of *Y. pseudotuberculosis*.

The identical plasmid profiles of the isolates from the patients, soil, sand, and cat strongly support the conjecture that *Y. pseudotuberculosis* serotypes 1b and 3 can be transmitted to humans through environmental substances such as water, soil, and sand contaminated by the feces of cats infected with these serotypes.

*Y. pseudotuberculosis* infections in cats were reported by Mair et al. (13). The infected cats showed clinical symptoms of anorexia, vomiting, and severe diarrhea. *Y. pseudotuberculosis* serotype 1b (cat and dog) and 6 (dog) strains, present at $1.2 \times 10^7$, $6.7 \times 10^6$, and $6.7 \times 10^6$ cells per g, respectively, were isolated from 1 (0.3%) of 359 diarrheal cats and from 2 (0.5%) of 395 diarrheal dogs from 1985 to 1988 in Shimane Prefecture (H. Fukushima and M. Gomyoda, submitted for publication). Moreover, *Y. pseudotuberculosis* strains were isolated from 1.3 to 6.3% of apparently healthy dogs and cats (5, 18). The maximum amount of these organisms in the rectum of an apparently healthy dog was $2.0 \times 10^6$ cells per g, although these organisms could be isolated from apparently healthy cats only by postenrichment culturing (5). In this study, two pieces of cat feces from which *Y. pseudotuberculosis* was isolated from postenrichment culturing were dry and hard. All these findings taken together strongly suggest that domestic pets such as cats and dogs can become an important source of infection with *Y. pseudotuberculosis*, regardless of the clinical condition.

*Y. pseudotuberculosis* serotype 4a and 5a strains isolated from one rodent showed negative reactions for virulence-associated phenotypic markers and lacked the virulence plasmid. Fukushima et al. (3) reported that avirulent *Y. pseudotuberculosis* serotype 5a and 6 strains were present in rodents in Shimane Prefecture. These findings suggested that avirulent strains of *Y. pseudotuberculosis* are present in wild animals like rodents.

**DISCUSSION**

This report seems to be the first evidence that *Y. pseudotuberculosis* can be transmitted to humans via water contaminated by bacteria in the feces of a cat. A 1-year-old boy was infected with *Y. pseudotuberculosis* serotypes 1b and 3 and a 3-year-old boy was infected with *Y. pseudotuberculosis* serotype 1b after drinking water from water puddles in a garden of their housing unit. These serotype 1b and 3 strains of *Y. pseudotuberculosis* were simultaneously isolated from soil from the dried-up puddles and sand in the sandbox collected on day 17 after this episode but never from the parents and older brother, household parakeet, or city drinking water on days 22 and 23 after this episode. These results strongly suggest that the water in the puddles was the source of the infection.

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**LITERATURE CITED**


3. Fukushima, H., M. Gomyoda, K. Shiozawa, S. Kaneko, and M.


