Short Communication

Turtle-Associated Salmonella Infections in Kanagawa, Japan

Toshiro Kuroki*, Kumiko Ito, Tomoe Ishihara, Ichiro Furukawa, Akiko Kaneko, Yu Suzuki, Junji Seto, and Tsutomu Kamiyama

1Department of Microbiology, 2Department of Planning and Information, Kanagawa Prefectural Institute of Public Health, Kanagawa 253-0087; 3Department of Clinical Laboratory, Yamagata Prefectural Central Hospital, Yamagata 990-2292; 4Department of Microbiology, Yamagata Prefectural Institute of Public Health, Yamagata 990-0031; and 5Kamiyama Pediatric Clinic, Kanagawa 250-0004, Japan

SUMMARY: In this paper, we examine 2 case reports for different reptile-related Salmonella enterica subspecies enterica serotypes. In case 1, a 5-year-old boy presented with gastroenteritis caused by S. enterica subspecies enterica serovar Poona. The suspected source of infection was a turtle kept at the patient’s home. In case 2, a 4-year-old boy presented with gastroenteritis caused by S. enterica subspecies enterica serovar Abony. The Pulsed-field gel electrophoresis analysis suggested that a tortoise kept at the patient’s home was the source of infection. This paper presents a review of the literature and an examination of cases regarding turtle-associated salmonellosis in Japan.

Reptiles, including turtles, are well-known reservoir hosts for Salmonella and are a major source of human salmonellosis (1,2). People can contact salmonellosis through direct or indirect contact with turtles. Salmonella can cause mild or severe diarrhea, gastroenteritis, and sometimes a serious infection (e.g., meningitis or septicemia) (3). Young children, elderly people, or people with immunodeficiency disorders are at a high risk for developing a severe Salmonella infection; however, young children are more likely to get salmonellosis from turtles due to inappropriate handling (4–6).

Two cases of turtle-associated salmonellosis in children were reported in Japan in 2007 and 2008. In the first case, reported in September 2007, a pet turtle was suspected as the source of Salmonella infection. In the second case, reported in March 2008, a pet tortoise was confirmed as the source by pulsed-field gel electrophoresis (PFGE).

Case 1: a 5-year-old boy was admitted to the hospital on September 30, 2007 after experiencing diarrhea for 2 days. The patient experienced watery diarrhea (5 to 6 times a day), abdominal pain, tenesmus, vomiting, anorexia, fever (39.5°C), and mild dehydration. The patient received a 2-day course of intravenous fluids and a 5-day course of fosfomycin. The illness resolved after 5 days.

To determine the presence of Salmonella, stool cultures inoculated onto SS agar plates (Eiken Chemical, Tokyo, Japan) and ES Salmonella agar II plates (Eiken Chemical) were tested at our laboratory for gastroenteritis causative agents. Plates were incubated at 36°C for 20 h. Suspected colonies were characterized biochemically on triple sugar iron agar, sulfur indole motility agar, and lysine media. Salmonella were confirmed serologically with O and H antisera (Denka Seiken, Tokyo, Japan). Salmonella enterica subspecies enterica serovar Poona was isolated. The family of the patient reported that they had been keeping an aquatic turtle as a pet at their home. It is likely that the turtle was the source of the infection; however, no further information and no specimen from the turtle was available for analysis.

Case 2: a 4-year-old boy presented to the hospital on March 31, 2008 after experiencing diarrhea for 3 days. The patient experienced watery diarrhea 3 to 4 times a day. No abdominal pain or fever was observed. The patient received intravenous fluids and a 5-day course of fosfomycin. The illness resolved after 5 days.

A stool specimen from the patient was cultured as described above and yielded S. enterica subspecies enterica serovar Abony. Salmonella was not isolated from stool specimens from the parents. The family had purchased a spur-thighed tortoise (Testudo graeca) at a pet shop one year earlier and kept it as a pet at their home. Stool specimens from the tortoise also yielded S. enterica subspecies enterica serovar Abony.

PFGE with BlnI and XbaI (Takara Bio, Inc., Shiga, Japan) was conducted on the S. enterica subspecies enterica serovar Abony isolates with a CHEF-DRIII system (Bio-Rad Laboratories, Tokyo, Japan). The PFGE analysis revealed that the patterns of the S. enterica subspecies enterica serovar Abony isolates from the patient and the tortoise were almost identical (Fig. 1) suggesting that the pet tortoise may have been the source of Salmonella infection in case 2.

The patient’s father usually handled the tortoise. The enclosure and related materials were washed in the kitchen sink or the bathroom. The parents reported that the patient sometimes handled the tortoise to play with and the parents were aware that aquatic turtles were Salmonella reservoir hosts; however, they were unaware that other reptiles, including terrestrial tortoises, could...
also carry *Salmonella*. Furthermore, pet shop personnel did not inform the parents that tortoises could be a source of *Salmonella* infection.

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method with BBL Sensi-discs (Becton, Dickinson and Company, Tokyo, Japan) and Mueller-Hinton II agar plates (Becton, Dickinson and Company). Discs containing the following antibiotics were used: ampicillin (10 μg), cefotaxime (30 μg), fosfomycin (50 μg), tetracycline (30 μg), gentamicin (10 μg), kanamycin (30 μg), streptomycin (10 μg), chloramphenicol (30 μg), nalidixic acid (30 μg), norfloxacin (10 μg), ciprofloxacin (5 μg), and sulfamethoxazole/trimethoprim (23.75/1.25 μg). In accordance with the Clinical and Laboratory Standards Institute criteria, the results were scored as susceptible, intermediate, or resistant. *Escherichia coli* ATCC25922 was used as the quality control strain. The isolate of *S. enterica* subspecies *enterica* serovar Poona from case 1 as well as the isolate of *S. enterica* subspecies *enterica* serovar Abony from case 2 and from the patient’s tortoise were sensitive to all antibiotics tested.

*S. enterica* subspecies *enterica* serovar Poona, a common reptile-related *Salmonella* serotype, is frequently isolated from turtles (1,2), including red-eared sliders (*Trachemys scripta elegans*) (7,8). Aquatic turtles, in particular, may become a source of *S. enterica* subspecies *enterica* serovar Poona and may cause sporadic cases or outbreaks (3,9). For example, Kaneko et al. described 8 sporadic cases and 1 outbreak of *S. enterica* subspecies *enterica* serovar Poona infection in Yamagata between 2005 and 2007 (10). Isolates from these cases were divided into 2 PFGE types by *Xba*I digestion and 3 types by *Bln*I digestion. Consequently, 3 clusters were found among the isolates. The first cluster was formed by isolates from 2 sporadic cases that occurred in 2007 and the PFGE pattern of the cluster was identical with that of the isolate from case 1 in the present study (lane 1 and 2 in Fig. 2). The result suggests that these cases are epidemiologically linked and a clone of *S. enterica* subspecies *enterica* serovar Poona has been widely disseminated throughout Japan. However, patient information regarding turtle contact in the 2007 Yamagata sporadic cases was not available. Nonetheless, previous reports examining reptile-associated salmonellosis have suggested that the direct reptile contact is not necessary for *Salmonella* transmission (11). Therefore, people without direct exposure to turtles are also at risk for turtle-associated salmonellosis (6). The second cluster consisted of isolates from 1 sporadic case and 1 outbreak that were associated with aquatic turtles kept at patients’ homes (lane 3 in Fig. 2). The third cluster included 5 sporadic cases that occurred in a community (lane 4 in Fig. 2). Although the PFGE analysis suggested that these cases were linked to a common source of infection, the source was not identified.

Human cases of turtle-associated salmonellosis have been reported in Japan since 1976 (Table 1) (10,12–23). Patient isolates of *S. enterica* subspecies *enterica* have included 14 serotypes including serovars Litchfield, Paratyphi B, Poona, Saintpaul, and Typhimurium. Almost all cases have involved children 6 years old or younger and gastroenteritis has been the predominant symptom; however, severe illness such as bacteremia and meningitis have also occurred (13,18,20,22). For example, in the first reports of turtle-associated salmonellosis in Japan, 2 patients developed gastroenteritis due to different *S. enterica* subspecies *enterica* serovars (Muenchen and Typhimurium) after contact with red-eared sliders (12). One of the patients also developed nephritis. Furthermore, a report from Hokkaido, Japan demonstrated that 24 cases of salmonellosis were associated with pet animals, such as red-eared sliders, tortoises, and crayfish (13). In addition, a patient with *S. enterica* subspecies *enterica* serovar Urbana showed neurologic symptoms and resulting sequelae (18). The majority of cases reviewed in this study were linked to red-eared sliders. Red-eared sliders are the most popular pet turtles worldwide as well as in Japan. The majority of turtle farms are located in Louisiana, US and produce approximately 2,500,000 red-eared sliders each year (24). Approximately 100,000 to 200,000 turtles are imported from the US into Japan annually (25). Two outbreaks and a sporadic case were associated with Reever’s turtles (*Chinemys reevesii*) (15) and terrestrial tortoises (22,23), respectively. *S. enterica* subspecies *enterica* serovar Poona was isolated in both instances.
A pulsed-field gel electrophoresis pattern of *Salmonella enterica* subspecies enterica serovar Poona isolates cleaved with restriction enzyme *Xba*I and *Bln*I. Lane 1, isolate from the case 1 patient; lane 2, isolate from a sporadic case of salmonellosis in Yamagata; lane 3, isolate from an outbreak of turtle-associated salmonellosis in Yamagata; lane 4, isolate from a sporadic case of salmonellosis occurred in a community in Yamagata; lane M, DNA size standard *S. enterica* subspecies enterica serovar Braenderup H9812.
Table 1. Summary of published cases of turtle-associated salmonellosis in Japan

<table>
<thead>
<tr>
<th>Yr</th>
<th>Salmonella enterica serotype</th>
<th>Turtle species</th>
<th>Age of patient</th>
<th>Diagnosis</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1976</td>
<td>S. Muenchen</td>
<td>Red-eared slider</td>
<td>5 yr</td>
<td>Gastroenteritis</td>
<td>Hiroshima</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>S. Typhimurium</td>
<td>Red-eared slider</td>
<td>5 yr</td>
<td>Gastroenteritis, nephritis</td>
<td>Hokkaido</td>
<td>13</td>
</tr>
<tr>
<td>1984</td>
<td>S. Paraphyph B</td>
<td>Red-eared slider</td>
<td>70 yr</td>
<td>Gastroenteritis</td>
<td>Fukuoka</td>
<td>14</td>
</tr>
<tr>
<td>1985</td>
<td>S. Itami</td>
<td>Reeve’s turtle</td>
<td>2 yr</td>
<td>Gastroenteritis</td>
<td>Ishikawa</td>
<td>15</td>
</tr>
<tr>
<td>1987</td>
<td>S. Litchfield</td>
<td>Red-eared slider</td>
<td>10 mo</td>
<td>Gastroenteritis</td>
<td>Aomori</td>
<td>17</td>
</tr>
<tr>
<td>1992</td>
<td>S. Litchfield</td>
<td>Red-eared slider</td>
<td>5 yr</td>
<td>Gastroenteritis, bacteraemia, neurologic symptom</td>
<td>Wakayama</td>
<td>18</td>
</tr>
<tr>
<td>2000</td>
<td>S. Urbana</td>
<td>Red-eared slider</td>
<td>2 mo</td>
<td>Gastroenteritis</td>
<td>Akita</td>
<td>19</td>
</tr>
<tr>
<td>2005</td>
<td>S. Paraphyph B</td>
<td>turtleistry</td>
<td>6 yr</td>
<td>Gastroenteritis, bacteraemia</td>
<td>Chiba</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>S. Braenderup</td>
<td>Red-eared slider</td>
<td>15 mo</td>
<td>Meningitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>S. Schleissheim</td>
<td>Red-eared slider</td>
<td>6 yr</td>
<td>Gastroenteritis</td>
<td>Nagasaki</td>
<td>21</td>
</tr>
<tr>
<td>2005-2006</td>
<td>S. Poona</td>
<td>Red-eared slider etc.</td>
<td>—</td>
<td>Gastroenteritis</td>
<td>Yamagata</td>
<td>10</td>
</tr>
<tr>
<td>2006</td>
<td>S. Poona</td>
<td>African spurred tortoise</td>
<td>7 mo</td>
<td>Gastroenteritis, bacteraemia</td>
<td>Niigata</td>
<td>22</td>
</tr>
<tr>
<td>2007</td>
<td>S. Poona</td>
<td>Aquatic turtle3)</td>
<td>5 yr</td>
<td>Gastroenteritis</td>
<td>Kanagawa</td>
<td>This study</td>
</tr>
<tr>
<td>2008</td>
<td>S. Abony</td>
<td>Spur-thighed tortoise</td>
<td>4 yr</td>
<td>Gastroenteritis</td>
<td>Kanagawa</td>
<td>This study</td>
</tr>
<tr>
<td>2009</td>
<td>S. Poona</td>
<td>Terrestrial tortoise5)</td>
<td>—</td>
<td>Gastroenteritis</td>
<td>Shizuoka</td>
<td>23</td>
</tr>
</tbody>
</table>

2): An outbreak in an elementary school including 162 cases in children.
3): including 3 sporadic cases between 2005 and 2006.
4): An outbreak in day-care centers including 9 cases in children.
5): Species of turtles were not identified.

It should be noted that, although the parents of the patient in case 2 were aware that aquatic turtles might carry Salmonella, they were not aware that terrestrial tortoises could also carry Salmonella. It is important that pet shop owners, veterinarians, and public health authorities provide appropriate information regarding Salmonella among aquatic turtles as well as terrestrial tortoises. Pet owners should recognize that both aquatic turtles and terrestrial tortoises harbor Salmonella and employ basic hygiene measures to reduce the risk of turtle-associated Salmonella infection.

Conflict of interest None to declare.

REFERENCES
19. Onuma S, Kudo M, Endo Y, et al. Two cases of turtle associated...


