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Summary

We present case reports of salmonellosis with reptile-related serotypes of *Salmonella enterica* subspecies *enterica*. The first case was a 5-year-old boy with gastroenteritis caused by *Salmonella enterica* subspecies *enterica* serovar Poona. A turtle, which was suspected as the source of infection, was kept at the patient’s home. The second case was a 4-year-old boy with gastroenteritis caused by *S. enterica* subspecies *enterica* serovar Abony. The PFGE analysis suggested that a tortoise, which was kept at the patient’s home, was the source of infection. We review the literature on turtle-associated salmonellosis in Japan in tandem with the turtle-associated salmonellosis we studied.

Reptiles including turtles are well-known reservoirs for *Salmonella* and are an important source of human salmonellosis (1,2). People get salmonellosis by direct or indirect contact with turtles. *Salmonella* can cause mild or severe diarrhea, gastroenteritis, and sometimes a serious infection, such as meningitis or septicemia (3). Young children, elderly people or people with immunodeficiency disorders are included in a high risk group for severe *Salmonella* infection. Among people in a high risk group, young children are more likely to get salmonellosis from turtles because they handle turtles inappropriately (4, 5, 6).

We had 2 cases of turtle-associated salmonellosis in children in 2007 and 2008. The first case occurred in September 2007 and a pet turtle was
suspected as a source of *Salmonella* infection. The second case appeared in March 2008 and was confirmed to be associated with a pet tortoise by pulsed-field gel electrophoresis.

A boy (case 1) aged 5 years was admitted to a hospital with diarrhea of 2 days duration on 30 September 2007. The patient had watery diarrhea 5 to 6 times a day, abdominal pain, tenesmus, vomiting, anorexia, fever of 39.5°C and mild dehydration. The patient received intravenous fluids for 2 days and was placed on a 5-day course of fosfomycin. The illness resolved after 5 days.

A stool specimen was tested at our laboratory (KPIPH) for the presence of causative agents of gastroenteritis using stool culture and was inoculated onto SS agar plates (Eiken Chemical, Tokyo, Japan) and ES *Salmonella* agar II plates (Eiken Chemical, Tokyo, Japan) to detect *Salmonella*. Plates were incubated at 36°C for 20 hours. Suspected colonies were characterized biochemically on triple sugar iron agar, sulfur indole motility agar and lysine medium. *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 have been keeping an aquatic turtle as a pet at their home, so that a turtle is likely the source of the infection. However, no further information and no specimen from the turtle was available.

A boy (case 2) aged 4 years was taken to a hospital with diarrhea of 3 days duration on 31 March 2008. The patient had watery diarrhea 3 to 4 times a day. No abdominal pain and fever were observed. The patient received intravenous fluids and was placed on a 5-day course of fosfomycin. The
illness resolved after 5 days.

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

Pulsed-field gel electrophoresis (PFGE) with *BlnI* and *XbaI* (Takara Bio, Inc., Shiga, Japan) was carried out 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 be a source of *Salmonella* infection for case 2 patient.

A father of the patient had usually handled the tortoise. The tortoise's enclosure and other stuff had been washed in the kitchen sink and the bathroom. The parents reported that the patient sometimes handled the tortoise to play with and the parents were aware that aquatic turtles were reservoirs for *Salmonella* but were unaware that other reptiles including terrestrial tortoises could carry *Salmonella*. Pet shop personnel did not inform the parents that tortoises could be a source of *Salmonella* infection.

The antimicrobial susceptibility test was performed based on the method of Kirby-Bauer disc diffusion susceptibility test using BBL Sensi-disc susceptibility test discs (Becton, Dickinson and Company, Tokyo, Japan) on Mueller-Hinton II agar plates (Becton, Dickinson and Company, Tokyo,
Discs containing the following antibiotics were used: ampicillin (10 μg), cefotaxime (30 μg), fosfomycin (50 μg), tetracycline (30 μg), gentamicin (10 μg), kanamicin (30 μg), streptomycin (10 μg), chloramphenicol (30 μg), nalidixic acid (30 μg), norfloxacine (10 μg), ciprofloxacine (5 μg), and Sulfamethoxazole/trimethoprim (23.75/1.25 μg). The results were scored as susceptible, intermediate, or resistant, according to Clinical and Laboratory Standards Institute criteria. Escherichia coli ATCC25922 was used as quality control strain. An isolate of S. enterica subspecies enterica serovar Poona from the case 1 patient and 2 isolates of S. enterica subspecies enterica serovar Abony from the case 2 patient and the tortoise that the patient’s family had kept, were sensitive to all antibiotics tested.

S. enterica subspecies enterica serovar Poona is usually recognized as one of reptile-related Salmonella serotypes and is frequently isolated from turtles (1,2), such as red-eared sliders (Trachemys scripta elegans) (7, 8). Aquatic turtles may become a source of S. enterica subspecies enterica serovar Poona, and cause sporadic cases or outbreaks (3, 9). Kaneko et al. described 8 sporadic cases and an outbreak of S. enterica subspecies enterica serovar Poona infection in Yamagata between 2005 and 2007 (10). Isolates from these cases were divided into two PFGE types by XbaI digestion and three types by BlnI digestion. Consequently, three clusters were found among the isolates. The first cluster was formed by isolates from two sporadic cases occurred in 2007. The PFGE pattern of the cluster was identical with that of the isolate from the case 1 of the present study (lane 1 and 2 in Fig. 2). The result suggested that these cases were epidemiologically linked and a clone of S. enterica subspecies enterica
serovar Poona distributed widely in Japan. Information of patients of the sporadic cases in Yamagata in 2007 on contact with turtles was not available. Reptile-associated salmonellosis previously reported suggested that the direct reptile contact is not necessary for Salmonella transmission (11). People without direct turtle exposure are at risk for turtle-associated salmonellosis (6). The second cluster consisted of isolates from a sporadic case and an outbreak that were associated with aquatic turtles which were 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). The PFGE analysis suggested that these cases were linked to a common source of infection, although a source was not identified.

The human cases of turtle-associated salmonellosis were reported since 1976 in Japan (Table 1) (10, 12–23). S. enterica subspecies enterica isolated from patients included 14 serotypes such as S. enterica subspecies enterica serovars Litchfield, Paratyphi B, Poona, Saintpaul, and Typhimurium. Almost all cases involved children of 6 years or younger. Gastroenteritis was the predominant symptom but severe illness such as bacteraemia and meningitis occurred (13, 18, 20, 22). The first report of turtle-associated salmonellosis in Japan showed that 2 patients with S. enterica subspecies enterica serovar Muenchen or Typhimurium had contact with red-eared sliders and developed gastroenteritis (12). One of the patients also had nephritis. The report from Hokkaido demonstrated that 24 cases of salmonellosis were associated with pet animals, such as red-eared sliders, a tortoise, and a crayfish (13). The patient with S. enterica subspecies enterica serovar Urbana showed neurologic symptoms and subsequent
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 in Japan and worldwide. The turtle farms in Louisiana, the United States, where most of the turtle farms are located, produce approximately 2,500,000 red-eared sliders each year (24). Approximately 100,000 to 200,000 turtles are imported from the United States into Japan annually (25). Two outbreaks and a sporadic case were associated with Reeve’s turtles (Chinemys reevesii) (15) and terrestrial tortoises (22, 23), from which S. enterica subspecies enterica serovar Poona was isolated.

S. enterica subspecies enterica serovar Abony is a rare serotype of S. enterica subspecies enterica, and is not frequently isolated from human salmonellosis. Only 6 (0.03%) among 19,077 Salmonella isolates from human salmonellosis were determined as S. enterica subspecies enterica serovar Abony in Japan between 2000 and 2010 (26). Nineteen (0.25%) out of 7,313 cases were caused by S. enterica subspecies enterica serovar Abony between 1990 and 2000 in Sweden, and 2 cases among 19 cases of S. enterica subspecies enterica serovar Abony infection were associated with turtles (2). Severe turtle-associated salmonellosis caused by S. enterica subspecies enterica serovar Abony was reported in Belgium, where S. enterica subspecies enterica serovar Abony was isolated from 3 (0.009%) out of 35,021 cases of Salmonella infection between 2003 and 2007 (27).

Spur-thighed tortoises are one of the most popular pet tortoises and originate in areas around the Mediterranean Sea. Previous reports demonstrated that spur-thighed tortoises frequently harbored Salmonella spp., and that S. enterica subspecies enterica isolates from spur-thighed
tortoises included 23 serotypes (28–32). Accordingly, spur-thighed tortoises should be considered as an important source of *Salmonella* for humans. Among the serotypes isolated from the tortoises, *S. enterica* subspecies *enterica* serovar Abony was one of the most prevalent serotypes (29, 30). Turtle-associated salmonellosis is characterized that children are likely to be a high risk group, because small turtles can be handled easily by children and are small enough to be placed in the mouth (4, 5, 6). Parents are likely to think that turtles are safer animals than lizards and snakes. The effects of regulation and education on salmonellosis from pet reptiles including turtles are documented. In the United States, sales of small turtles with a carapace length less than 4 inches have been banned since 1975, because turtles commonly carry *Salmonella* and have higher risk of human salmonellosis in young children than other reptiles (5). The prohibition had prevented an estimated 100,000 cases of turtle-associated salmonellosis in children in 1980 (33). The regulation is supposed to be the most effective public health action to prevent turtle-associated salmonellosis (5).

The regulation prohibiting small turtles in Sweden had effect to reduce turtle-associated *Salmonella* infection (2). When Sweden joined the European Union in 1996 and the ban was repealed, the number of cases of turtle-associated salmonellosis increased. Along with the regulation, a public education campaign had an effect on preventing reptile-associated salmonellosis. Providing appropriate information to the public about the risk of reptile-associated salmonellosis decreased the number of *Salmonella* infections (2).

It should be noted that the parents of the case 2 did not know that
terrestrial tortoises could carry *Salmonella*, even though they knew that aquatic turtles might have *Salmonella*. It is important that pet shop owners, veterinarians and public health authorities should provide appropriate information on the carriage of *Salmonella* among aquatic turtles and terrestrial tortoises and the risk of *Salmonella* infection from turtles. Pet owners should know that both aquatic turtles and terrestrial tortoises harbor *Salmonella* and practice basic hygiene measures to reduce the risk of turtle-associated *Salmonella* infection.

Conflict of interest  None to declare.

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free-living spur-thighed tortoises (*Testudo graeca*) in central western


Figure legends

Fig. 1 Pulsed-field gel electrophoresis pattern of *Salmonella enterica* subspecies *enterica* serovar Abony isolates cleaved with restriction enzyme *XbaI* and *BlnI*. Lane 1, isolate from the case 2 patient; lane 2, isolate from a spur-thighed tortoise kept at a patient house; lane M, DNA size standard *Salmonella enterica* subspecies *enterica* serovar Braenderup H9812.
Fig. 2  Pulsed-field gel electrophoresis pattern of *Salmonella enterica* subspecies *enterica* serovar Poona isolates cleaved with restriction enzyme *XbaI* and *BlnI*. 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 *Salmonella enterica* subspecies *enterica* serovar Braenderup H9812.
<table>
<thead>
<tr>
<th>Year</th>
<th>Salmonella enterica</th>
<th>Turtle species</th>
<th>Age of patient</th>
<th>Diagnosis</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>1976</td>
<td>S. Muenchen</td>
<td>Red-eared slider</td>
<td>5 yr</td>
<td>Gastroenteritis</td>
<td>Hiroshima</td>
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<td>Red-eared slider</td>
<td>5 yr</td>
<td>Gastroenteritis, nephritis</td>
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<td>1981</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1984</td>
<td>S. Paratyphi B</td>
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<td>70 yr</td>
<td>Gastroenteritis</td>
<td>Fukuoka</td>
<td>14</td>
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<tr>
<td>1985</td>
<td>S. Itami</td>
<td>Reeve’s turtle</td>
<td>-</td>
<td>Gastroenteritis</td>
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<tr>
<td>1987</td>
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<td>2 yr</td>
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<td>Fukushima</td>
<td>16</td>
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<td>1992</td>
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<td>Gastroenteritis</td>
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<td>2000</td>
<td>S. Urbana</td>
<td>Red-eared slider</td>
<td>5 yr</td>
<td>Gastroenteritis, bacteraemia, neurologic symptom</td>
<td>Wakayama</td>
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<td>2003</td>
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<td>2 mo</td>
<td>Gastroenteritis</td>
<td>Akita</td>
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<td>6 yr</td>
<td>Gastroenteritis, bacteraemia</td>
<td>Chiba</td>
<td>20</td>
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<tr>
<td>2005</td>
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<td>Red-eared slider</td>
<td>15 mo</td>
<td>Meningitis</td>
<td></td>
<td></td>
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<td>2005</td>
<td>S. Schleissheim</td>
<td>Red-eared slider</td>
<td>6 yr</td>
<td>Gastroenteritis</td>
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<td>Shizuoka</td>
<td>23</td>
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3): including 3 sporadic cases in children.
4): An outbreak in day-care centers including 9 cases in children.
5): Species of turtles were not identified.
Fig. 2