Prevalence of *Salmonella enterica* subspecies *enterica* in red-eared sliders *Trachemys scripta elegans* retailed in pet shops in Japan

Toshiro Kuroki, Tomoe Ishihara, Naoki Nakajima, Ichiro Furukawa, and Yumi Une

Received: April 13, 2018. Accepted: September 11, 2018
Published online: September 28, 2018
DOI:10.7883/yoken.JJID.2018.140

Advance Publication articles have been accepted by JJID but have not been copyedited or formatted for publication.
Prevalence of *Salmonella enterica* subspecies *enterica* in red-eared sliders *Trachemys scripta elegans* retailed in pet shops in Japan

Toshiro Kuroki 1* †, Tomoe Ishihara 1, Naoki Nakajima 1, Ichiro Furukawa 1, Yumi Une 2 ‡

1 Department of Microbiology, Kanagawa Prefectural Institute of Public Health, 1-3-1 Shimomachiya, Chigasaki, Kanagawa 253-0087, Japan

2 Department of Pathology, School of Veterinary Medicine, Azabu University, Fuchinobe 1-17-71, Chuou-ku, Sagamihara, Kanagawa 252-5201 Japan

*Corresponding author: Toshiro Kuroki
Department of Microbiology, Kanagawa Prefectural Institute of Public Health 1-3-1 Shimomachiya, Chigasaki, Kanagawa 253-0087, Japan
E-mail: kuroki.gcg3@pref.kanagawa.jp
Tel: +81-467-83-4400, Fax: +81-467-4457

† Current address  Department of Parasitology, Faculty of Veterinary Medicine, Okayama University of Science, Ikoinooka 1-3, Imabari, Ehime 974-8555, Japan
e-mail: t-kuroki@vet.ous.ac.jp
Tel: +81-898-52-9086, Fax: +81-898-9022

‡ Current address Department of Pathology, Faculty of Veterinary Medicine, Okayama University of Science, Ikoinooka 1-3, Imabari, Ehime 974-8555, Japan

Key words: Salmonella, turtle, prevalence, genetic characteristics

Running title: Prevalence of Salmonella in turtles
Domestic author information

黒木俊郎†，石原ともえ，中嶋直樹，古川一郎，宇根有美

1 神奈川県衛生研究所微生物部
〒253-0087 神奈川県茅ヶ崎市下町屋1-3-1

2 麻布大学獣医学部獣医学科病理学教室
〒252-0206 神奈川県相模原市中央区淵野辺1-17-71

† 現所属 岡山理科大学獣医学部獣医学科医動物学講座
〒794-8555 愛媛県今治市いこいの丘1-3

‡ 現所属 岡山理科大学獣医学部獣医学科病理学講座
〒794-8555 愛媛県今治市いこいの丘1-3
Summary
We investigated the prevalence of *Salmonella* in 227 small red-eared sliders (*Trachemys scripta elegans*) from 2006 to 2008. One hundred thirty turtles (57.3%) were positive for *S. enterica* subsp. *enterica*. Twenty-two serotypes including *S. Montevideo*, *S. Newport*, *S. Pomona*, *S. Braenderup*, *S. Sandiego*, and *S. Litchfield* were identified. *Salmonella* strains with closely related PFGE patterns were isolated from several shops located in different areas over 2006 to 2008. Antimicrobial resistance was detected among strains of *S. Montevideo*, *S. Newport*, *S. Braenderup*, *S. Sandiego*, and *S. Litchfield*. The relatedness of antimicrobial resistance and PFGE profiles was not observed. PFGE patterns of *S. Poona* strains isolated in 2006 and 2008 and the causative strains of turtle-associated salmonellosis in 2006 were identical. These results revealed a high prevalence of *Salmonella enterica* subsp. *enterica* in red-eared sliders retailed in Japan. In addition, genetically closely related strains of turtle-associated *Salmonella* were repeatedly introduced into Japan over the study period, and were distributed in a wide area of Japan. These *Salmonella* strains present a risk of a widely disseminated outbreak of turtle-associated salmonellosis.
Introduction

Non-typhoid *Salmonella enterica* subsp. *enterica* often causes gastroenteritis in humans. However, infection may develop into severe invasive infections, particularly in high risk groups such as immunocompromised patients, elderly individuals, infants and children under 5 years old. The organisms are ubiquitous in the natural environment, and people usually acquire *Salmonella* by ingestion of contaminated food. The other infection route is direct or indirect contact with infected animals (1). Among animals, pet reptiles, including turtles, play important roles, because reptiles frequently harbor *Salmonella* in their intestine (2, 3). The investigations of turtle-associated salmonellosis in the 1960s and 1970s in the United States demonstrated that over 100 cases of turtle-associated salmonellosis occurred after the first report in 1963, children under 10 years old were at high risk, and small turtles posed a special risk of infection (2). In 1975, the sale of small turtles was banned in the United States for turtles with a carapace length of less than 4 inches. This ban followed increased public health concerns over turtle-associated salmonellosis, and was effective in reducing an estimated 100,000 cases of turtle associated salmonellosis annually in the United States (3).

Although the sale of small turtles has been banned in the United States, the regulation excludes the export trade. Millions of small turtles per year, mostly red-eared sliders (*Trachemys scripta elegans*), are exported from the United States into
other countries (4). These small turtles were an important source of turtle-associated salmonellosis worldwide (4–8). Hundreds of thousands of small turtles are exported from the United States to Japan every year (9). Two cases of salmonellosis associated with red-eared sliders in children were first documented in 1975 in Japan (8). As subsequent reports of turtle-associated salmonellosis arose (10,11), the role of pet turtles as a source of salmonellosis was identified in Japan as well as other countries. Approximately 40 years after the first case, several cases of reptile-associated salmonellosis have been reported in Japan (12–14).

Limited information exists as to the frequency of Salmonella infection in red-eared sliders retailed in Japan. The aim of the present study was to determine the prevalence of Salmonella enterica subsp. enterica in red-eared sliders for sale in pet shops in Japan. In addition, the genetic characteristics of Salmonella strains isolated from turtles were analyzed to determine how Salmonella strains were geographically distributed over time in Japan. This information can be used to inform turtle-associated Salmonella control measures.

**Materials and methods**

Collection of samples

Two hundred twenty-seven juvenile red-eared sliders were purchased from 29 pet shops from 2006 to 2008, consisting of 13 shops in the eastern area (the Tohoku and
Kanto regions) and 16 shops in the western area (the Kinki and Kyushu regions) of Japan. The numbers of turtles tested in each year were 94, 28, and 105. The number of turtles purchased from each shop ranged from 2 to 22. Turtle weight ranged from 5.6 to 11.8g (average: 8.7g) and length of the carapace ranged from 29.7 to 40.2mm (average: 34.2mm). Turtles were sacrificed by decapitation and dissected. Intestine, liver and yolk sac were removed aseptically from turtles, and were individually cut into small pieces with sterile scissors.

**Salmonella** isolation and identification

The samples were suspended in 5 ml of buffered peptone water (BPW) (Oxoid, Tokyo, Japan). After incubation at 36 °C for 20–22 hours, 1ml of BPW culture was transferred to 10ml of Hajna-tetrathionate broth (Eiken Chemical, Tokyo, Japan). The broth was incubated at 42 °C for 22 hours, then one loopful culture from each tube was inoculated onto plates of *Shigella-Salmonella* agar (Eiken Chemical) and ES *Salmonella* agar II (Eiken Chemical). These plates were incubated at 36 °C for 20–22 hours and three suspicious colonies morphologically similar to *Salmonella* spp. from each plate were subcultured for biological examinations. Biochemical characteristics were examined on triple sugar iron medium (Eiken Chemical), Sulfate Indole Motility medium (Eiken Chemical), lysine medium (Eiken Chemical) and Voges-Proskauer semisolid medium (Eiken Chemical). The subspecies of *Salmonella* isolates was
confirmed with ID 20 (Nissui Pharmaceutical, Tokyo, Japan) and API-20E (bioMérieux, Tokyo, Japan). Serotyping of *Salmonella* isolates was accomplished with commercial O and H antisera (Denka Seiken, Tokyo, Japan). The serotypes were designated according to the Kauffmann-White scheme (15).

Isolates of *S*. Poona


Antimicrobial susceptibility testing

Strains of *S*. Montevideo, *S*. Newport, *S*. Sandiego, *S*. Litchfield, *S*. Braenderup, and *S*. Pomona were tested for antimicrobial resistance. Susceptibilities to antibiotics were determined by the Kirby-Bauer (K-B) method according to the Clinical and Laboratory Standards Institute standards (16). *Salmonella* isolates from 20 hour cultures were suspended in sterile phosphate buffered saline. Suspension was inoculated with a sterile swab onto a Mueller-Hinton agar plate (BD, Tokyo, Japan). Sensi-Disc Susceptibility Test Discs (BD) with 12 different antibiotics were placed with Sensi-Disc Dispensers (BD) on the inoculated surface. Antibiotics analyzed were ampicillin (10 µg), cefotaxime (10 µg), fosfomycin (50 µg), tetracycline (30 µg), gentamicin (10 µg), kanamycin (30 µg), streptomycin (10 µg), chloramphenicol (30 µg), and spectinomycin (30 µg).
µg), nalidixic acid (30 µg), norfloxacin (10 µg), ciprofloxacin (5 µg), and sulfamethoxazole/trimethoprim (23.75/1.25 µg). Plates were incubated at 36 °C for 18–20 hours. Zones of inhibition were measured and the results were interpreted according to Clinical and Laboratory Standards Institute criteria (16). *Escherichia coli* strain ATCC25922 was used as the quality control strain.

Pulsed-field gel electrophoresis analysis

PFGE analysis was carried out on selected *Salmonella* isolates using the protocol as previously described (17). The PFGE profiles were analyzed using Bionumerics software version 6.6.3 (Applied Maths Sint-Martens-Latem, Belgium). Dendrograms for PFGE patterns were generated based on the unweighted pair group method using average linkages similarity cluster analysis performed with Dice coefficients, with a 1.0% band position tolerance for PFGE.

Results

Prevalence of *Salmonella* in red-eared sliders

*Salmonella* was isolated from 189 turtles (83.3%) purchased from 28 pet shops (96.6%) (Table 1). One hundred thirty turtles (57.3%) from 23 pet shops (79.3%) were positive for the presence of *S. enterica* subsp. *enterica* (Table 1). Proportion of *Salmonella* in turtles purchased from each shop ranged from 0–100% (average:
A total of 22 serotypes of *S. enterica* subsp. *enterica* were detected from the turtles tested. Certain turtles harbored more than one serotype. The most frequently detected serotype was Montevideo, followed by Newport, Pomona, Braenderup, Sandiego, and Litchfield (Table 1). Other serotypes isolated from the turtle samples were O:4 (B): Lagos, Paratyphi B(Java), Saintpaul, Schwarzengrund, and Typhimurium; O:7 (C1): Bareilly, Oranienburg, Oslo, and Thompson; O:8 (C1-C2): Narashino, and Muenchen; O:9 (D1): Berta, O:3,10 (E1): Anatum; O:16 (I): Barranquilla; O:13 (G): Poona; and O:18 (K): Cerro.

Genetic characteristics of *Salmonella* isolates

To determine the genetic characteristics of *Salmonella* isolates detected from the turtles, the isolates of *S.* Montevideo, *S.* Newport, *S.* Pomona, *S.* Braenderup, *S.* Sandiego, and *S.* Litchfield were analyzed by PFGE, and the dendrogram of these serotypes was generated with BioNumerics software (Fig. 1).

*S.* Montevideo was isolated from both areas of Japan, but only in 2006. *S.* Montevideo isolates formed two clusters with a similarity cutoff of 90% and two isolates did not form clusters (Fig.1A). All isolates in cluster MO2 were resistant to gentamicin, kanamycin, and tetracycline. Two isolates in cluster MO2 showed an identical PFGE pattern, and one isolate was detected from the eastern area and another
was from the western area.

*S. Newport* was detected in 2006, 2007 and 2008. *S. Newport* isolates formed one cluster with a similarity cutoff of 90% (Fig. 1B). Eight isolates did not form clusters, and the PFGE patterns of these isolates were diverse.

*S. Pomona* was isolated only in 2008, and formed one cluster consisting of 4 isolates from the eastern area and 3 isolates from the western area (Fig. 1C). Among the 7 isolates, 4 isolates exhibited an identical PFGE pattern. No isolates of *S. Pomona* were resistant to the antibiotics tested.

*S. Braenderup* was isolated only in 2006. Three from the eastern area and 2 from the western area, and all isolates showed an identical PFGE pattern (data not shown).

*S. Sandiego* and *S. Litchfield* were isolated in 2006 and 2008. Among 9 isolates of *S. Sandiego*, 7 isolates detected from both the eastern and western areas formed one cluster with a similarity cutoff of 90% (Fig. 1D). *S. Litchfield* formed 3 clusters with a similarity cutoff of 90% (Fig. 1E). Cluster LI2 included isolates from only the eastern area, whereas 5 isolates from the western area formed clusters LI1 and LI3.

*S. Poona* was isolated from turtles purchased from 2 shops located in Kanagawa Prefecture in the eastern area in 2006 and 2008. The PFGE profile of the isolates from turtles purchased in these 2 shops is consistent with that of *S. Poona* isolated in Yamagata Prefecture in 2006 (Fig. 2).
Antimicrobial resistance

Antimicrobial resistant strains were detected from S. Montevideo, S. Newport, S. Sandiego, S. Litchfield, and S. Braenderup (Fig. 1). Two strains of S. Braenderup were resistant to streptomycin: one isolated from a turtle from a shop in the eastern area, and one from the western area. The one S. Braenderup strain from the western area was also resistant to sulfamethoxazole/trimethoprim.

Discussion

Red-eared sliders (Trachemys scripta elegans) are raised on turtle farms in the United States and are exported worldwide (18). Red-eared sliders are frequently found to carry Salmonella (19, 20). Cases of red-eared slider-associated salmonellosis have been reported in the United States and other countries, including Canada, Spain and Japan (3–8).

The present study showed a high prevalence of S. enterica subsp. enterica in small red-eared sliders sold in pet shops in Japan. The results of this study agree with previous studies indicating a high prevalence of Salmonella in red-eared sliders in import traders or pet shops (4, 6, 21). A study in Puerto Rico detected Salmonella from all 18 shops investigated (4). The prevalence of Salmonella in 28 lots of turtle eggs that were exported from the United States to Canada ranged from 8.3 to 83.3% (6). A study in Brazil on the prevalence of Salmonella in pet reptiles revealed that Salmonella was...
detected from 14 out of 34 (41.2%) imported red-eared sliders (21).

It is well known that a number of *Salmonella* serotypes are associated with reptiles, and they are a part of the normal intestinal flora (5). In the present study, *S.* Montevideo, *S.* Newport, *S.* Pomona, *S.* Braenderup, *S.* Sandiego, and *S.* Litchfield were isolated from turtles from pet shops in Japan. These serotypes were detected previously in reptiles (22), and were associated with outbreaks of turtle-associated human salmonellosis in the United States (5).

The results of the PFGE analysis indicate that genetically closely related *Salmonella* isolates were found from turtles sold in the eastern and western areas in Japan and/or in different sample collection years (Table 1, Fig. 1). These results suggest that the sources of *Salmonella* existed continuously, genetically closely related strains of *Salmonella* carried by turtles were repeatedly introduced into Japan, and strains derived from the same source of *Salmonella* were disseminated over wide areas in Japan. Red-eared sliders are imported from the United States to Japan, and are widely traded in Japan through pet wholesalers to retailers. *Salmonella* may have colonized turtle farms in the United States, and the turtle farms may be the source of *Salmonella*. Surveys of *Salmonella* contamination on turtle farms have shown that *Salmonella* was consistently detected from eggs and hatchlings of turtles at turtle farms and in the environment of turtle farms, including pond water and nest soil (20, 23). The regulations of Louisiana mandate that *Salmonella* in turtle eggs be eliminated by
treatment with antibiotics at turtle farms (24). However, antibiotic treatment is unable to eradicate *Salmonella* from turtle eggs. Survivors spread among turtles packed in containers during shipment, and thus turtles become contaminated with *Salmonella* (20).

In the present study, the PFGE patterns of *S*. Poona strains isolated from turtles purchased in Kanagawa in 2006 and 2008 and from a human outbreak of turtle-associated salmonellosis in Yamagata in 2006 (14) were indistinguishable. These results suggest that turtles harboring the outbreak strain of *S*. Poona were widely distributed in Japan. While there have been no previously reported disseminated outbreaks of turtle-associated salmonellosis in Japan, many multistate outbreaks of *Salmonella* infection caused by pet turtles have been reported in the United States since 2006 (5). The surveillance system of infectious diseases in Japan needs to focus on reptile-related salmonellosis to detect a disseminated outbreak of the illness.

While proportion of resistant strains was not high, antimicrobial resistance against tetracycline, gentamicin, kanamycin, streptomycin, and sulfamethoxazole/trimethoprim was detected among *Salmonella* isolates. The linkage between antimicrobial resistance and the PFGE profiles was not observed in this study. The reason may be the low number of *Salmonella* isolates analyzed of each serotype, and the low prevalence of resistant strains. Further studies would be needed to determine the relatedness of drug resistance and genetic types of *Salmonella*. 
The present study indicates the potential risk of human salmonellosis from pet turtles, and suggests that some control measures should be taken to decrease the risk of salmonellosis from pet turtles in Japan. Informing the public about the risk of salmonellosis from reptiles has been reported to decrease the number of reptile-related salmonellosis cases (22). Thus, control measures based upon advices provided by the Ministry of Health, Labour and Welfare of Japan, may reduce the risk of salmonellosis from pet turtles (25). These recommendations are: 1) to wash hands thoroughly with soap and water after handling turtles, 2) to avoid keeping turtles in homes with children, elderly individuals, or people with weakened immune systems, 3) to keep turtles from roaming freely in homes, the living area, or the food preparation area, 4) to avoid using kitchen sinks to wash enclosures.

Another measure for preventing turtle-associated salmonellosis includes instituting regulations to restrict sales of turtles. A previous report showed that the regulations issued in the United States in 1975 to ban the sales of turtles decreased the number of reptile-associated salmonellosis cases (3). However, the risks and cases of turtle-associated salmonellosis have recently been increasing, because of illegal sales of small turtles (5). Although banning sales of turtles has been effective in reducing the number of salmonellosis cases, effect of the regulations is limited. Thus, effective combination of education and regulations may be needed.
Acknowledgement

This study was supported by a Grant-in-Aid from Ministry of Health, Labour and Welfare, Japan. The authors appreciate Department of Microbiology, Yamagata Prefectural Institute of Public Health for providing S. Poona outbreak-associated strains.

Conflict of interest None to declare.

References


5. Bosch S, Tauxe R, Behravesh CB. Turtle-associated salmonellosis, United States,


22. de Jong B, Andersson Y, Ekdahl K. Effect of regulation and education on


Figure legends

Figure 1 Combined dendrogram of pulsed-field gel electrophoresis patterns with XbaI and BlnI for Salmonella Montevideo (A), Newport (B), Pomona (C), Sandiego (D), and Litchfield (E). Cluster analysis was performed with Bionumerics using the Dice correlation coefficient and the unweighted pair group method using arithmetic average clustering algorithm. GM: gentamycin, KM: kanamycin, SM: streptomycin, ST: sulfamethoxazole/trimethoprim.

Figure 2 Pulsed-field gel electrophoresis pattern of Salmonella enterica subspecies enterica serovar Poona isolates digested with restriction enzyme XbaI and BlnI. Lane 1, the isolate from a turtle sold at shop T in Kanagawa in 2006; lane 2, the isolate from a turtle-associated salmonellosis patient in Yamagata in 2006; lane 3, the isolate from turtle enclosure water in the patient’s house in Yamagata in 2006; lane 4, the isolate from a turtle sold at shop R in Kanagawa in 2008; lane M, DNA size standard Salmonella enterica subspecies enterica serovar Braenderup H9812 digested with XbaI.
Table 1 Detection of *Salmonella* from turtles and by shops

<table>
<thead>
<tr>
<th>Area</th>
<th>No. of samples</th>
<th>Salmonella spp.</th>
<th>Salmonella enterica subsp. enterica</th>
<th>Serotypes</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Montevideo</td>
<td>Newport</td>
<td>Pomona</td>
<td>Braenderup</td>
<td>Sandiego</td>
<td>Litchfield</td>
<td>Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turtles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>227</td>
<td>189 (83.3)</td>
<td>130 (57.3)</td>
<td>25 (11.0)</td>
<td>23 (10.1)</td>
<td>14 (6.2)</td>
<td>8 (3.5)</td>
<td>7 (3.1)</td>
<td>7 (3.1)</td>
<td>66 (29.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eastern</td>
<td>81</td>
<td>67 (82.7)</td>
<td>48 (59.3)</td>
<td>8 (9.9)</td>
<td>9 (11.1)</td>
<td>11 (13.6)</td>
<td>2 (2.5)</td>
<td>2 (2.5)</td>
<td>1 (1.2)</td>
<td>22 (27.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western</td>
<td>146</td>
<td>122 (83.6)</td>
<td>82 (56.1)</td>
<td>17 (11.6)</td>
<td>14 (9.6)</td>
<td>3 (2.1)</td>
<td>6 (4.1)</td>
<td>5 (3.4)</td>
<td>6 (4.1)</td>
<td>44 (30.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shops</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>28 (96.6)</td>
<td>23 (79.3)</td>
<td>8 (27.6)</td>
<td>7 (24.1)</td>
<td>6 (20.7)</td>
<td>5 (17.2)</td>
<td>5 (17.2)</td>
<td>4 (13.8)</td>
<td>12 (41.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eastern</td>
<td>13</td>
<td>12 (92.3)</td>
<td>10 (76.9)</td>
<td>3 (23.1)</td>
<td>3 (23.1)</td>
<td>5 (38.5)</td>
<td>2 (15.4)</td>
<td>2 (15.4)</td>
<td>1 (7.7)</td>
<td>6 (46.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western</td>
<td>16</td>
<td>16 (100)</td>
<td>13 (81.3)</td>
<td>5 (31.3)</td>
<td>4 (25.0)</td>
<td>1 (6.3)</td>
<td>3 (18.8)</td>
<td>3 (18.8)</td>
<td>3 (18.8)</td>
<td>6 (37.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1): Number of turtles tested

2): Parentheses show percentage.

3): Number of shops
<table>
<thead>
<tr>
<th>Strain</th>
<th>Year</th>
<th>Area</th>
<th>Antibiotic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSAL 3765</td>
<td>2006</td>
<td>western</td>
<td></td>
</tr>
<tr>
<td>KSAL 3745</td>
<td>2006</td>
<td>western</td>
<td></td>
</tr>
<tr>
<td>KSAL 3706</td>
<td>2006</td>
<td>western</td>
<td></td>
</tr>
<tr>
<td>KSAL 3707</td>
<td>2006</td>
<td>western</td>
<td></td>
</tr>
<tr>
<td>KSAL 3711</td>
<td>2006</td>
<td>eastern</td>
<td></td>
</tr>
<tr>
<td>KSAL 3797</td>
<td>2006</td>
<td>western</td>
<td></td>
</tr>
<tr>
<td>KSAL 3723</td>
<td>2006</td>
<td>western</td>
<td></td>
</tr>
<tr>
<td>KSAL 3802</td>
<td>2006</td>
<td>eastern</td>
<td>GM,KM,TC</td>
</tr>
<tr>
<td>KSAL 3672</td>
<td>2006</td>
<td>eastern</td>
<td>GM,KM,TC</td>
</tr>
<tr>
<td>KSAL 3708</td>
<td>2006</td>
<td>eastern</td>
<td>GM,KM,TC</td>
</tr>
<tr>
<td>KSAL 3803</td>
<td>2006</td>
<td>western</td>
<td></td>
</tr>
<tr>
<td>KSAL 3648</td>
<td>2006</td>
<td>eastern</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain</th>
<th>Year</th>
<th>Area</th>
<th>Antibiotic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSAL 4680</td>
<td>2008</td>
<td>western</td>
<td></td>
</tr>
<tr>
<td>KSAL 4572</td>
<td>2008</td>
<td>eastern</td>
<td></td>
</tr>
<tr>
<td>KSAL 4798</td>
<td>2008</td>
<td>eastern</td>
<td></td>
</tr>
<tr>
<td>KSAL 4793</td>
<td>2008</td>
<td>eastern</td>
<td></td>
</tr>
<tr>
<td>KSAL 4693</td>
<td>2008</td>
<td>western</td>
<td></td>
</tr>
<tr>
<td>KSAL 4832</td>
<td>2008</td>
<td>western</td>
<td>GM</td>
</tr>
<tr>
<td>KSAL 4545</td>
<td>2008</td>
<td>eastern</td>
<td></td>
</tr>
<tr>
<td>KSAL 4510</td>
<td>2008</td>
<td>eastern</td>
<td></td>
</tr>
<tr>
<td>KSAL 4514</td>
<td>2008</td>
<td>eastern</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1  Combined dendrogram of pulsed-field gel electrophoresis patterns with XbaI and BlaI for Salmonella Montevideo (A), Newport (B), Pomona (C), Sandiego (D), and Litchfield (E). Cluster analysis was performed with Bionumerics using the Dice correlation coefficient and the unweighted pair group method using arithmetic average clustering algorithm. GM: gentamycin, KM: kanamycin, SM: streptomycin, ST: sulfamethoxazole/trimethoprim.
Fig. 2  Pulsed-field gel electrophoresis pattern of *Salmonella enterica* subspecies *enterica* serovar Poona isolates cleaved with restriction enzyme *XbaI* and *BlnI*. Lane 1, the isolate from a turtle sold at shop T in Kanagawa in 2006; lane 2, the isolate from a turtle-associated salmonellosis patient in Yamagata in 2006; lane 3, the isolate from turtle enclosure water in the patient's house in Yamagata in 2006; lane 4, the isolate from a turtle sold at shop R in Kanagawa in 2008; lane M, DNA size standard *Salmonella enterica* subspecies *enterica* serovar Braenderup H9812.