Jpn. J. Infect. Dis., 68, 333–337, 2015

Short Communication

Turtle-Associated *Salmonella* Infections in Kanagawa, Japan

Toshiro Kuroki[1]*, Kumiko Ito[2], Tomoe Ishihara[1], Ichiro Furukawa[1], Akiko Kaneko[3], Yu Suzuki[4], Junji Seto[4], and Tsutomu Kamiyama[5]

[1]*Department of Microbiology,* [2]*Department of Planning and Information, Kanagawa Prefectural Institute of Public Health, Kanagawa 253-0087;*
[3]*Department of Clinical Laboratory, Yamagata Prefectural Central Hospital, Yamagata 990-2292;*
[4]*Department of Microbiology, Yamagata Prefectural Institute of Public Health, Yamagata 990-0031; and*
[5]*Kamiyama 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 *Bln*I and *Xba*I (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

Received October 29, 2014. Accepted December 8, 2014.
J-STAGE Advance Publication March 13, 2015.
DOI: 10.7883/yoken.JJID.2014.490
*Corresponding author: Mailing address: Department of Microbiology, Kanagawa Prefectural Institute of Public Health, Shimomachiya 1-3-1, Chigasaki, Kanagawa 253-0087, Japan. Tel: +81-0467-83-4400, Fax: +81-0467-83-4457, E-mail: kuroki.gcg3@pref.kanagawa.jp

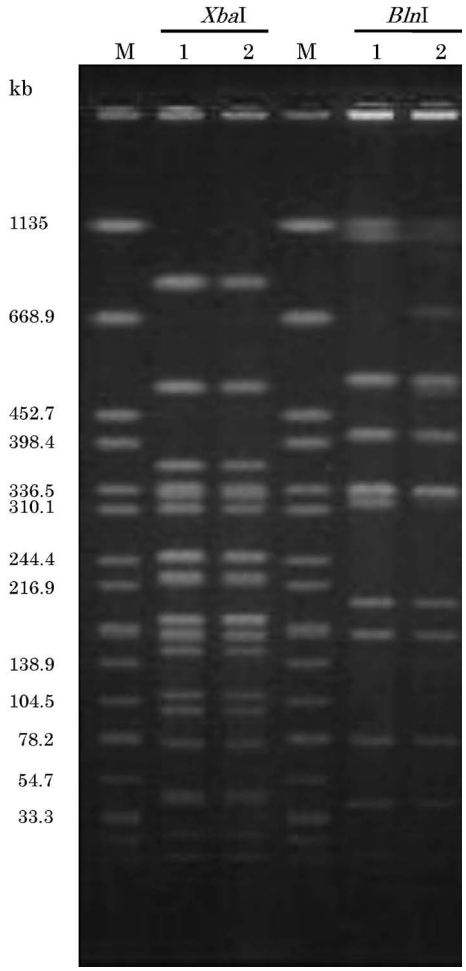

Fig. 1. Pulsed-field gel electrophoresis pattern of *Salmonella enterica* subspecies *enterica* serovar Abony isolates cleaved with restriction enzyme *Xba*I and *Bln*I. Lane 1, isolate from the case 2 patient; lane 2, isolate from a spur-thighed tortoise kept at a patient house; lane M, DNA size standard *S. enterica* subspecies *enterica* serovar Braenderup H9812.

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 Reeve's turtles (*Chinemys reevesii*) (15) and terrestrial tortoises (22,23), respectively. *S. enterica* subspecies *enterica* serovar Poona was isolated in both instances.

S. enterica subspecies *enterica* serovar Abony is a rare

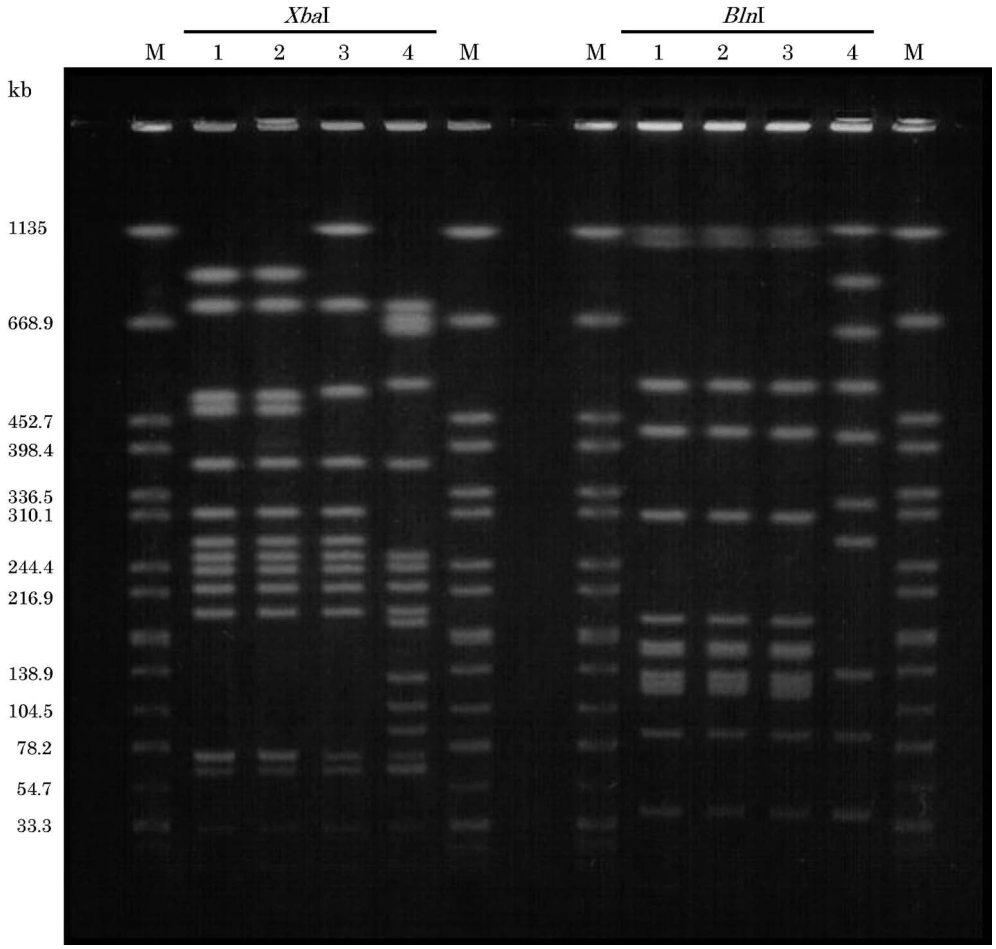

Fig. 2. Pulsed-field gel electrophoresis pattern of *Salmonella enterica* subspecies *enterica* serovar Poona isolates cleaved with restriction enzyme *Xba*I and *Bln*I. Lane 1, isolate from the case 1 patient; lane 2, isolate from a sporadic case of salmonellosis in Yamagata; lane 3, isolate from an 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.

serotype of *S. enterica* subspecies *enterica*, and is not frequently isolated in cases of human salmonellosis. Between 2000 and 2010, only 6 (0.03%) of 19,077 *Salmonella* isolates from human salmonellosis cases were determined as *S. enterica* subspecies *enterica* serovar Abony in Japan (26). In Sweden, 19 (0.25%) out of 7,313 cases were caused by *S. enterica* subspecies *enterica* serovar Abony between 1990 and 2000, and 2 of these 19 cases 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 in 3 (0.009%) of 35,021 cases of *Salmonella* infection between 2003 and 2007 (27).

Spur-thighed tortoises are one of the most popular types of pet tortoises and originate in areas around the Mediterranean Sea. Previous reports have demonstrated that spur-thighed tortoises frequently harbor *Salmonella* and that *S. enterica* subspecies *enterica* isolates from these tortoises include 23 serotypes (28–32). Accordingly, spur-thighed tortoises should be considered a major source of *Salmonella* for humans. Among the serotypes isolated from the tortoises, *S. enterica* subspecies *enterica* serovar Abony was one of the most prevalent (29,30). Although parents often think that turtles are safer for children than lizards and snakes, children are at a high risk of contracting turtle-associated salmonellosis from small turtles because they can be easily handled and placed in the mouth (4–6). However, the effects of regulation and education have been shown to be effective in preventing salmonellosis from pet reptiles, including turtles. For example, given that turtles commonly carry *Salmonella* and are more likely to transmit salmonellosis to young children than other reptiles, sales of small turtles with a carapace length less than 4 inches have been banned in the US since 1975 (5). The ban prevented an estimated 100,000 cases of turtle-associated salmonellosis in children in 1980 (33). The regulation may be the most effective public health action to prevent turtle-associated salmonellosis (5).

Similar regulations prohibiting small turtles in Sweden reduced turtle-associated *Salmonella* infections in that country (2). However, when Sweden joined the European Union in 1996, the ban was repealed, and the number of cases of turtle-associated salmonellosis increased. In addition to regulations, a public health education campaign providing appropriate information to the public about the risk of reptile-associated salmonellosis also helped to decrease the number of reptile-associated *Salmonella* infection (2).

Table 1. Summary of published cases of turtle-associated salmonellosis in Japan

Yr	*Salmonella enterica* serotype	Turtle species	Age of patient	Diagnosis	Location	Reference
1976	*S.* Muenchen	Red-eared slider	5 yr	Gastroenteritis	Hiroshima	12
	S. Typhimurium	Red-eared slider	5 yr	Gastroenteritis, nephritis		
1977–1981[1)]	*S.* Typhimurium, *S.* Java, *S.* Saintpaul, *S.* Heidelberg, *S.* Hartford	Red-eared slider etc.	—	Gastroenteritis, bacteraemia, acute hemiplegia	Hokkaido	13
1984	*S.* Paratyphi B	Red-eared slider	70 yr	Gastroenteritis	Fukuoka	14
1985[2)]	*S.* Itami	Reeve's turtle	—	Gastroenteritis	Ishikawa	15
1987	*S.* Litchfield	Red-eared slider	2 yr	Gastroenteritis	Fukushima	16
1992	*S.* Litchfield	Red-eared slider	10 mo	Gastroenteritis	Aomori	17
2000	*S.* Urbana	Red-eared slider	5 yr	Gastroenteritis, bacteraemia, neurologic symptom	Wakayama	18
2003	*S.* Saintpaul	turtle[5)]	2 mo	Gastroenteritis	Akita	19
		turtle[5)]	3 yr	Gastroenteritis		
2005	*S.* Paratyphi B	Red-eared slider	6 yr	Gastroenteritis, bacteraemia	Chiba	20
	S. Braenderup	Red-eared slider	15 mo	Meningitis		
2005	*S.* Schleissheim	Red-eared slider	6 yr	Gastroenteritis	Nagasaki	21
2005–2006[3)]	*S.* Poona	Red-eared slider etc.	—	Gastroenteritis	Yamagata	10
2006	*S.* Poona	African spurred tortoise	7 mo	Gastroenteritis, bacteraemia	Niigata	22
2007	*S.* Poona	Aquatic turtle[5)]	5 yr	Gastroenteritis	Kanagawa	This study
2008	*S.* Abony	Spur-thighed tortoise	4 yr	Gastroenteritis	Kanagawa	This study
2009[4)]	*S.* Poona	Terrestrial tortoise[5)]	—	Gastroenteritis	Shizuoka	23

[1)]: including 24 sporadic cases in children between 1977 and 1981.
[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

1. Woodward DL, Khakhria R, Johnson WM. Human salmonellosis associated with exotic pets. J Clin Microbiol. 1997;35:2786-90.
2. de Jong B, Andersson Y, Ekdahl K. Effect of regulation and education on reptile-associated salmonellosis. Emerg Infect Dis. 2005;11:398-403.
3. Public Health Laboratory Service (PHLS). Baby dies of *Salmonella* poona infection linked to pet reptile. Commun Dis Rep CDR Wkly. 2000;10:161.
4. Centers for Disease Control and Prevention (CDC). Turtle-associated salmonellosis in humans—United States, 2006–2007. MMWR Morb Mortal Wkly Rep. 2007;56:649-52.
5. Centers for Disease Control and Prevention (CDC). Multistate outbreak of human *Salmonella* infections associated with exposure to turtles—United States, 2007–2008. MMWR Morb Mortal Wkly Rep. 2008;57:69-72.
6. Centers for Disease Control and Prevention (CDC). Multistate outbreak of human *Salmonella* Typhimurium infections associated with pet turtle exposure—United States, 2008. MMWR Morb Mortal Wkly Rep. 2010;59:191-6.
7. Shane SM, Gilbert R, Harrington KS. *Salmonella* colonization in commercial pet turtles (*Pseudemys scripta elegans*). Epidemiol Infect. 1990;105:307-16.
8. D'Aoust JY, Daley E, Crozier M, et al. Pet turtles: a continuing international threat to public health. Am J Epidemiol. 1990;132:233-8.
9. Centers for Disease Control and Prevention (CDC). Eight multistate outbreaks of human *Salmonella* infections linked to small turtles. Available at <http://www.cdc.gov/salmonella/small-turtles-03-12/index.html>. Accessed October 7, 2014.
10. Kaneko N, Aoki T, Ohtani K, et al. PFGE analysis for *Salmonella* Poona strains isolated in Yamagata Prefecture. 34th annual meeting of the Yamagata Society of Public Health. March 5, 2008; Yamagata, Japan. Japanese.
11. Mermin J, Hoar B, Angulo FJ, et al. Iguanas and *Salmonella* Marina infection in children: a reflection of the increasing incidence of reptile-associated salmonellosis in the United States. Pediatrics. 1997;99:399-402.
12. Nakamori J, Miyazaki K, Nishio T, et al. Pet terrapin-linked salmonellosis; first proved cases in Japan and its epidemiology. Rinsho-to-Saikin. 1976;3:88-94. Japanese.
13. Fujita K, Murono K, Yoshioka H. Pet-linked salmonellosis. Lancet. 1981;2:525.
14. Murao T, Mako T, Nishimoto K. Red-eared slider turtle-associated *Salmonella enterica* serotype Paratyphi B gastroenteritis. Annual Report of Fukuoka City Institute for Hygiene and Environment. 1985;10:70-1. Japanese.
15. Serikawa T, Kimura N, Ishikawa S, et al. A mass outbreak of *Salmonella* itami infections. Annual Report of Ishikawa Research Laboratory for Public Health and Environment, 1986;23:329-31. Japanese.
16. Hirose M, Hirasawa K, Koguro Y, et al. Survey of pet turtles and their raising water for the contamination with *Salmonella*. Annual Report of Fukushima Institute of Public Health and Environmental Science. 1988;5:57-61. Japanese.
17. Kibayashi M. A case report of turtle-associated salmonellosis in infancy. Med J Aomori. 1992;37:288-91. Japanese.
18. Minami H, Yanagawa T, Kobayashi M, et al. A case of salmonellosis with severe neurological impairment caused by *Salmonella* Urbana (O30), which antigen is identical to the *Escherichia coli* O157 antigen. Infect Immun Childhood. 2000;12:19-22. Japanese.
19. Onuma S, Kudo M, Endo Y, et al. Two cases of turtle associated

gastroenteritis caused by *Salmonella* Saintpaul. Jpn J Pediatrics. 2005;58:2273-6. Japanese.
20. Nagano N, Oana S, Nagano Y, et al. A severe *Salmonella enterica* serotype Paratyphi B infection in a child related to a pet turtle, *Trachemys scripta elegans*. Jpn J Infect Dis. 2006;59:132-4.
21. Kaibu H, Iida K, Ueki S, et al. Salmonellosis of infants presumably originating from an infected turtle in Nagasaki, Japan. Jpn J Infect Dis. 2006;59:281.
22. Nishiwaki K, Iizuka T, Watanabe O, et al. An infant case of sepsis due to *S.* Poona infected presumably from a tortoise, May 2006—Niigata. Infect Agents Surveillance Rep. 2006;27:203-4.
23. Tuchiya Y, Hata N, Kato K, et al. Sporadic *Salmonella* Poona infection in nursery schools in Hamamatsu City. Infect Agents Surveillance Rep. 2010;31:105-7.
24. The Louisiana State University Agricultural Center. Louisiana summary agriculture and natural resources. Available at <http://www.lsuagcenter.com/agsummary/>. Accessed October 7, 2014.
25. Ministry of Finance Japan. Trade statistics of Japan. Available at <http://www.customs.go.jp/toukei/info/>. Accessed October 7, 2014.
26. Infectious Disease Surveillance Center, National Institute of Infectious Diseases. Isolation of bacteria in the past years, prefectural/municipal public health institutes and health centers, *Salmonella* serovars (not included *S.* Typhi & *S.* Paratyphi A). Available at <http://idsc.nih.go.jp/iasr/virus/pbacteria-e.html>. Accessed October 7, 2014.
27. Van Meervenne E, Botteldoorn N, Lokietek S, et al. Turtle-associated *Salmonella* septicaemia and meningitis in a 2-month-old baby. J Med Microbiol. 2009;58:1379-81.
28. Hidalgo-Vila J, Díaz-Paniagua C, de Frutos-Escobar C, et al. *Salmonella* in free living terrestrial and aquatic turtles. Vet Microbiol. 2007;119:311-5.
29. Hidalgo-Vila J, Díaz-Paniagua C, Ruiz X, et al. *Salmonella* species in free-living spur-thighed tortoises (*Testudo graeca*) in central western Morocco. Vet Rec. 2008;162:218-9.
30. Percipalle M, Giardina G, Lipari L, et al. *Salmonella* infection in illegally imported spur-thighed tortoises (*Testudo graeca*). Zoonoses Public Health. 2011;58:262-9.
31. Lecis R, Paglietti B, Rubino S, et al. Detection and characterization of *Mycoplasma* spp. and *Salmonella* spp. in free-living European tortoises (*Testudo hermanni*, *Testudo graeca*, and *Testudo marginata*). J Wildl Dis. 2011;47:717-24.
32. Giacopello C, Foti M, Annamaria Passantino A, et al. Serotypes and antibiotic susceptibility patterns of *Salmonella* spp. isolates from spur thighed tortoise *Testudo graeca* illegally introduced in Italy. Human Veterinary Med. 2012;4:76-80.
33. Cohen ML, Potter M, Pollard R, et al. Turtle-associated salmonellosis in the United States. Effect of Public Health Action, 1970 to 1976. JAMA. 1980;243:1247-9.