



## Prevalence of *Salmonella* spp. in pet turtles and their environment

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Pet turtles are known as a source of *Salmonella* infection to humans when handled in captivity. Thirty four turtles purchased from pet shops and online markets in Korea were examined to determine whether the turtles and their environment were contaminated with *Salmonella* spp. *Salmonella* spp. were isolated from fecal samples of 17 turtles. These isolates were identified as *S. enterica* through 16S rRNA gene sequencing. The isolation rate of *Salmonella* spp. from the soil and water samples increased over time. We concluded that a high percentage of turtles being sold in pet shops were infected with *Salmonella* spp., and their environments tend to become contaminated over time unless they are maintained properly. These results indicate that pet turtles could be a potential risk of salmonellosis in Korea.

**Keywords:** Prevalence, pet turtles, environment, *Salmonella* spp., salmonellosis

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*Salmonella* spp., as gram negative coccobacilli causing various diseases in both humans and animals, are some of the most important water-borne and food-borne pathogens worldwide.

The current view of *Salmonella* spp. taxonomy assigns the members of this genus to two species: *S. enterica* and *S. bongori*. *S. enterica* itself is divided into six subspecies, *enterica*, *salamae*, *arizonae*, *diarizonae*, *indica*, and *houtenae*, also known as subspecies I, II, IIIa, IIIb, IV, and VI, respectively. Members of the *Salmonella enterica* subspecies *enterica* are mainly associated with warm-blooded vertebrates and are usually transmitted by ingestion of food or water contaminated with infected feces [1].

Reptiles are asymptomatic carriers of *Salmonella* spp. infection, and they intermittently excrete these organisms in their feces [2-5]. However, humans show clinical symptoms when infected with *Salmonella* spp. such as acute gastroenteritis caused by *S. enterica* serovar

*Enteritidis* and *S. enterica* serovar *Typhimurium*, which are nontyphoidal salmonellosis [6-8]. After 6-48 hours of incubation, several days of clinical symptoms develop such as fever, headache, nausea, vomiting, stomachache, and diarrhea but typically patients recover in three days [9,10]. Therefore, reptiles constitute a potential source of zoonotic disease [11].

Human cases of salmonellosis are often associated with food, but frequently people become infected by handling infected animals such as infected turtles and contaminated materials such as water and soil [12,13]. Also, people who are immature or immune-compromised including babies, children under 5 years, pregnant women, elderly people and people with AIDS are prone to *Salmonella* infection, which results in clinical signs such as fever and diarrhea if they do not wash their hands after handling pet turtles [14,15]. In Korea, two patients visited Severance children's hospital in Seoul with a chief complaint of fever and were diagnosed with

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nontyphoidal salmonellosis [16].

As more people become interested in pet animals and the related business thrives, the number of aquariums, pet shops, online shops as well as pet cafes selling amphibians and reptiles has increased. Pet reptiles are known as a reservoir for potential zoonotic pathogens including viruses, bacteria and fungi, and bacteria in particular are excreted as normal flora in reptiles and amphibians resulting in the classification of their species as a high-risk group due to possible clinical signs from direct and indirect infection of immuno-compromised and elderly people, children and pregnant women [17].

The association between salmonellosis and pet turtles motivated a 1975 federal ban on the sale of turtles with a carapace of <10.2 cm (4 inches) in the United States [18,19]; however, here are no such regulations in Korea [20]. Since the bacteria are excreted through feces, environmental factors such as water and soil are also capable of transmitting the bacteria [21]. To prevent *Salmonella* spp. infection in baby turtles, the method of administering antibiotics before hatching is known; however, the effects seem temporary and can even cause antibiotic resistance [22,23]. In addition, the negligence of pet turtle owners in cleaning the cage and water increases the detection rate of *Salmonella* spp. Recently the rise in single-person households, increase in elderly households and low birthrate caused an increase in the number of households raising pet animals (dogs, cats, and exotic animals). As the affection between the owner and pet deepens, physical contact increases and allows for the risk of exposure to a pathogen [11].

The susceptibility of salmonellosis for children under 5 years is 5 times greater than adults [24,25]. For example, 39 children who attended a Komodo dragon (*Varanus komodoensis*) exhibit at the Denver Zoo in Colorado in 1996 became infected with *Salmonella* spp. though none touched the animals [26]. Therefore, families, nurseries, and schools raising turtles as pets must separate the turtles from children under 5 years and other immuno-compromised people. In particular, the purchase of turtles bigger than 4 inches and maintenance of a clean environment for raising the turtles is crucial. Pet shops must also inform their customers about the risk of infection through water even if the turtles are over 4 inches and emphasize the importance of cage cleanliness. Lastly, hospitals and health care providers must educate patients about the risks of pet turtles and emphasize the importance of hand-washing (<http://www.cdc.gov/>

salmonella/small-turtles-03-12). Therefore, Korea must enact such laws to prevent salmonellosis infection from small pet turtles.

We studied the isolation rate of *Salmonella* spp. from fecal samples of pet turtles purchased in pet shops as well as water and soil samples in their environment over time, and we used this to assess the risk of infection by pet turtles and their environment from a public health standpoint and provided information concerning the prevention of salmonellosis.

## Materials and Methods

### Purchase of pet turtles

Thirty four turtles of 6 commercially popular species were purchased through 9 pet shops and 8 online markets where are located in Seoul, Daejeon, Cheongju and Busan. The purchased turtles had an average weight of  $15\pm 2$  g, carapace diameter of  $40\pm 5$  mm, and were under 4 weeks of age.

Among the 34 turtles, 10 Chinese stripe-necked turtles (*Ocadia sinensis*), 8 yellow belly sliders (*Trachemys scripta scripta*), 6 river cooters (*Pseudemys concinna concinna*), 4 northern Chinese softshell turtles (*Pelodiscus maackii*), 3 western painted turtles (*Chrysemys picta belli*) and 3 common musk turtles (*Sternotherus odoratus*) were studied.

### Fecal sample collection

Each of the purchased turtles was placed in 500 mL beaker with 5 mL of sterilized distilled water for 24 hr. One mL of the distilled water containing the turtle's feces was taken as a fecal sample. In order to prevent *Salmonella* spp. transmission among individuals, poly-gloves were changed each time an animal was handled.

### Enrichment and isolation of *Salmonella* spp.

The samples were cultured according to the International Standard method ([http://www.iso.org/iso/catalogue\\_detail.htm?csnumber=29315](http://www.iso.org/iso/catalogue_detail.htm?csnumber=29315), 2002). The fecal samples were suspended in 5 mL of sterile saline, and 1 mL of the suspension was inoculated into 9 mL of tetrathionate broth (TTB, MB cell Ltd., Seoul, Korea) at 37°C for 24 hr. After incubation, the broth was mixed for 5 seconds with a Vortex agitator. Then one loopful from each tube was streaked onto a plate of selective media, brilliant green agar (BGA, MB cell Ltd., Seoul, Korea) and xylose lysine desoxycholate (XLD, MB cell Ltd.,

Seoul, Korea). These plates were incubated at 37°C for 24 hr. Colonies suspected of containing *Salmonella* spp. such as pink-white colonies on BGA and black colonies on XLD were inoculated and cultured in the other selective medium to isolate them.

#### Identification using 16S rRNA sequencing

All 17 strains of presumptive *Salmonella* spp. were identified using 16S rRNA sequencing with the universal primers 27F and 1492R in Cosmogenetech Co., Ltd. (Seoul, Korea).

#### Raising condition of pet turtles in cages

A total of 17 cages each containing 1-3 turtles of the same species from the same pet shop were managed; each cage had a slope made with soil and pebbles, 2 L of sterilized water made with a water filtration system and a canister filter. During this study, the change in isolation rate over time of *Salmonella* spp. in water, soil and skin samples was examined. Feeding of turtles followed the general husbandry method: Gammarus dried shrimp with calcium supplements (Samhotech Co., Ltd., Seoul, Korea) were fed twice a day while water temperature was kept within 26±2°C, pH 6.5-8.2, and 12 hr of photoperiod each day were maintained during the experiment.

#### Isolation rate of *Salmonella* spp. from environment over time

To study the presence of *Salmonella* spp. in the turtles' environments over time, water and soil samples were collected every 2 days from each of the 17 cages for 10 days. Five grams of soil from a turtle's rest or feeding area was obtained using a sterile spoon and incubated in 10 mL of TTB [27]. Ten milliliters of water were collected from each cage with a sterile pipette and suspended at room temperature for 10 min. Then 1 mL of the precipitate was inoculated into 9 mL of TTB. Those samples were incubated at 37°C for 24 hr in enrichment media. The method of enrichment and isolation was done by the same method as the fecal sample.

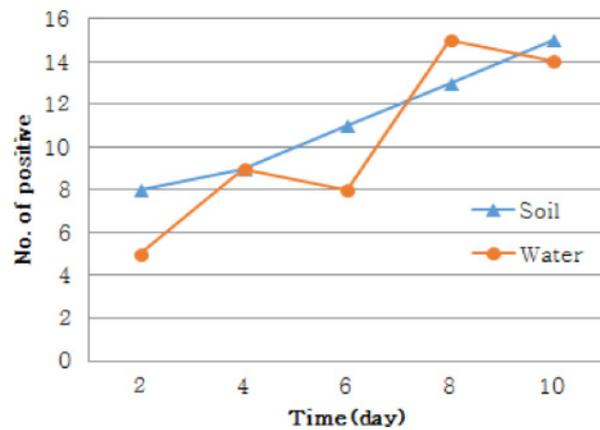
## Results

#### Isolation and identification of *Salmonella* spp. from fecal samples

*Salmonella* spp. were isolated from 17 (50.0%) of 34 turtle fecal samples (Table 1); positive samples were

**Table 1.** Isolation rate of *Salmonella* spp. by turtle species

Species	No. tested	No. positive	Isolation rate* (%)
<i>Ocadia sinensis</i>	10	8	80
<i>Trachemys scripta scripta</i>	8	3	37.5
<i>Pseudemys concinna concinna</i>	6	3	50
<i>Pelodiscus maackii</i>	4	3	75
<i>Chrysemys picta belli</i>	3	0	0
<i>Stemotherus odoratus</i>	3	0	0
Total	34	17	50



**Figure 1.** Number of the turtle's cages isolated *Salmonella* spp. from water and soil samples increased over time

from 8 of 10 Chinese stripe-necked turtles, 3 of 8 yellow belly sliders, 3 of 6 river cooters, 3 of 4 northern Chinese softshell turtles and no *Salmonella* spp. was isolated from the western painted turtles and common musk turtles. 16S rRNA sequencing results confirmed the isolate as *S. enterica*.

#### Isolation rate of *Salmonella* spp. from environment over time

As shown in Figure 1. below, the isolation rate of *Salmonella* spp. increased over time. In other words, on day 2 *Salmonella* spp. was isolated from five water samples and 8 soil samples; however, *Salmonella* spp. was isolated from 14 water samples and 15 soil samples on day 10. This demonstrates that most of the cage environments were contaminated with *Salmonella* spp. over time.

## Discussion

As more people become interested in pet animals and the related business thrives, the number of aquariums, pet shops, online shops as well as pet cafes selling

amphibians and reptiles has increased. Pet reptiles are known as a reservoir for potential zoonotic pathogens including viruses, bacteria and fungi, and bacteria in particular are excreted as normal flora in reptiles and amphibians resulting in the classification of their species as a high-risk group due to possible clinical signs from direct and indirect infection of immuno-compromised and elderly people, children and pregnant women [17].

The susceptibility of salmonellosis for children under 5 years is 5 times greater than adults [24,25]. For example, 39 children who attended a Komodo dragon exhibit at the Denver Zoo in Colorado in 1996 became infected with *Salmonella* spp. though none touched the animals [26]. Therefore, families, nurseries, and schools raising turtles as pets must separate the turtles from children under 5 years and other immuno-compromised people. In particular, the purchase of turtles bigger than 4 inches and maintenance of a clean environment for raising the turtles is crucial. Pet shops must also inform their customers about the risk of infection through water even if the turtles are over 4 inches and emphasize the importance of cage cleanliness. Lastly, hospitals and health care providers must educate patients about the risks of pet turtles and emphasize the importance of hand-washing. Therefore, Korea must enact such laws to prevent salmonellosis infection from small pet turtles.

In this study, *Salmonella* spp. was isolated from fecal samples of 17 of 34 turtles (50%). In similar studies, *Salmonella* spp. was isolated from 10.3% of turtles in zoo of Roma [7] and 6.61 and 6.38% of native and free-living turtles in parts of Spain [28]. In comparison, this study showed a higher isolation rate. However, *Salmonella* spp. was isolated from 72.2% of turtles in pet shops in Japan [29], a higher percentage than our study. This shows the possible horizontal transmission between turtles due to careless management of pet shops as well as raising many turtles in the same cage. Since *Salmonella* spp. shedding might be intermittent and stress-related [30,31], obtaining *Salmonella* spp. from wild turtles by single sampling will not be easy. From the results above, it can be concluded that domestic turtle raising and distribution has potential risk for *Salmonella* spp. infection.

In the present study, 16S rRNA sequencing of all isolates from fecal samples genetically confirmed it as *S. enterica*. Most cases of salmonellosis are caused by food infected with *S. enterica* have also been shown to be sources of infection to humans. Secreted proteins are of

major importance for the pathogenesis of infectious diseases caused by *S. enterica* [1].

As Figure 1. shows a gradual increase of *Salmonella* spp. isolation rate over time, most cages were gradually contaminated unless the water was changed and the environment was kept clean. Therefore, careless management of the raising environment such as failing to change the water and soil will result in *Salmonella* spp. contamination.

This study concluded that many small pet turtles distributed in Korea were infected with *Salmonella* spp. and improper management of their raising environments will increase the contamination of *Salmonella* spp. over time.

Although currently *S. enterica* has 6 subspecies, and each subspecies has associated 2500 serovars that differ by antigenic specificity, only about 50 serovars are isolated in any significant numbers as human or animal pathogens and they all belong to the subspecies *enterica*. The pathogenicity of most of the distinct serovars remains undefined and, even within the most common serovars, many questions remain to be answered regarding the interactions between the organism and the infected host [1,32]. In addition, further studies should focus on the pathogenicity to humans and antibiotic resistance of *Salmonella* subspecies and serovars obtained from this study.

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**Conflict of interests** The authors declare that there is no financial conflict of interests to publish these results.

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