

Dissemination of Zoonotic Antibiotic Resistant Serotypes of *Salmonella* by Caspian Pond Turtles, Golestan and Mazandaran Provinces, Iran



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Abstract

Background: *Salmonella* spp. are widespread zoonotic pathogens with economic importance for both humans and animals. They are categorized as the natural flora of the gastrointestinal tract of many reptiles. Human salmonellosis acquired from contact with reptiles is a well-recognized medical problem.

Objective: The frequency of *Salmonella* contamination in Caspian pond turtles was surveyed to evaluate the danger of exposure or disease risk for humans as these species are abundant around the villages in Golestan and Mazandaran provinces.

Materials and Methods: One hundred fifty fecal samples from Caspian pond turtles were tested by standard bacteriological methods and positive samples were serotyped. Antimicrobial susceptibility tests on isolated *Salmonella* strains were also performed.

Results: Out of 150 samples, 54 turtles were detected to be contaminated with *Salmonella*. Of the 54 *Salmonella* isolates, 38.8% (21/54) were serotyped as *S. typhimurium*; 35.1% (19/54) as *S. enterica* subsp. *enterica*; 9.2% (5/54) as *S. enterica* subsp. *salamae*; 9.2% (5/54) as *S. enterica* subsp. *arizona*; and 7.4% (4/54) as *S. enterica* subsp. *houtenae*. Female (28/80, 35%) and male (26/74, 35.1%) turtles showed equal incidence of *Salmonella* spp. contamination. Resistance was mostly observed against ampicillin (37%) followed by tetracycline (33.3%), nalidixic acid (7.4%), ciprofloxacin (5.5%), and cotrimoxazole (3.7%). The highest susceptibility was observed against gentamicin (100%) and trimethoprim (98.1%).

Conclusion: Our findings confirmed that people who are in close exposure to Caspian pond turtles and their feces are at the risk of *Salmonella* contamination. Accordingly, fundamental principles of hygiene should be applied in human contact with Caspian pond turtles. Furthermore, people should be educated about the *Salmonella* contamination which may occur through Caspian pond turtles.

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Introduction

Salmonella spp. are widespread zoonotic pathogens of economic importance both for humans and animals with high numbers of reservoirs including warm and cold blooded animals. Salmonellosis usually causes abdominal cramps and self-limiting diarrhea. However, serious complications may happen, particularly among children less than 10 years old, elderly people, and immunosuppressed patients.^{1,2}

Different species of reptiles have been introduced as *Salmonella* reservoirs without any sign of salmonellosis, although *Salmonella* may cause significant diseases in turtles.³⁻⁵ There is no information about the role of reptiles

in the *Salmonella* spp. transmission to human population in Iran, but according to the results of *Salmonella* surveillance in the United States during 1987–1997, 7 out of the top 20 serovars were reptile-associated *Salmonella* serovars.⁶

Despite presence of free living turtles in the rural areas of North of Iran, there is no information about the epidemiological situation regarding *Salmonella* spp.

The Caspian pond turtle, *Mauremys caspica*, belongs to the family Geoemydidae and is a medium-sized freshwater turtle that is widespread in most permanent freshwaters throughout the Middle East.⁷ In Iran, the Caspian pond turtle is widely distributed in the north

(Golestan and Mazandaran provinces), west, and south-west of the country.^{8,9}

Presence of many sources of freshwater in Golestan and Mazandaran provinces and their geographical situation make these provinces suitable for agricultural activities. In this regard, there are many villages in these two provinces in which Caspian pond turtles live around human houses and have high chance for exposure to humans.

The aim of current survey was to analyze the frequency of *Salmonella* contamination, serotypes of isolated *Salmonella*, and *Salmonella* antibiotic resistance patterns in Caspian pond turtles to estimate the possible health implications of *Salmonella* spp. transmission to humans and wild animals in Golestan and Mazandaran provinces.

Materials and Methods

Sampling

From 2015 to 2017, 150 apparently healthy Caspian pond turtles were randomly captured from the freshwaters around the villages of Golestan and Mazandaran provinces in North of Iran. All the villages were in the same climatic condition with different sources of freshwater such as pond and river. Size and sex of the turtles were documented.

Bacteriological Culture and Serotyping

Bacteriological analysis was performed on fecal samples. Each sample was enriched with Buffered Peptone Water (incubation was performed at 37°C for 16-20 hours). In the next step, culture medium was selectively enriched with Selenite F Broth. Then the suspected bacteria were transferred to Brilliant Green agar, Salmonella-Shigella agar, and Hektoen enteric agar and incubated at 37°C for 24-48 hours. Other screening tests such as gram-staining, motility, urease, catalase, and oxidase were also completed on all *Salmonella* suspected samples.

Positive samples were serotyped by commercial polyvalent anti-sera for somatic (O) and flagellar (H) antigens. The comprehensive antigenic formula was used according to the Kaufmann-White scheme.¹⁰

Antibiotic Resistance Pattern

All detected *Salmonella* spp. were analyzed for antibacterial resistance against 8 commonly used antibiotics by Kirby-Bauer disk diffusion method.¹⁰ Following antibiotics were used in this study: ampicillin (10), gentamicin (10), cotrimoxazole (25), trimethoprim (5), tetracycline (30), ciprofloxacin (5), nalidixic acid (30), and streptomycin (25). The results were categorized as susceptible, intermediate, or resistant as described by Wikler.¹¹

Results

From 150 Caspian pond turtles, 54 (36%) turtles were contaminated with *Salmonella* spp. Of the 54 *Salmonella* isolates, 38.8% (21/54) were serotyped as *S. typhimurium*; 35.1% (19/54) as *S. enteric* subsp. *enterica*; 9.2% (5/54) as *S. enterica* subsp. *salamae*; 9.2% (5/54) as *S. enterica* subsp.

arizona; and 7.4% (4/54) as *S. enteric* subsp. *houtenae*. None of the *Salmonella* positive turtles carried more than one serotype. Female (28/80, 35%) and male (26/74, 35.1%) turtles showed equal incidence of contamination with *Salmonella* spp.

Salmonella isolates were at least resistant or intermediate to one of the tested antibiotics. The results of the antibacterial resistance pattern are described in Table 1. All *Salmonella* isolates revealed susceptibility to a large number of the antimicrobial drugs tested. Resistance was mostly observed against ampicillin (37%) followed by tetracycline (33.3%), nalidixic acid (7.4%), ciprofloxacin (5.5%), and cotrimoxazole (3.7%). The highest susceptibility was observed against gentamicin (100%) and trimethoprim (98.1%).

Discussion

Caspian pond turtle is one of the most common turtle in the freshwater sources of Golestan and Mazandaran provinces, which is in contact with a wide range of wild species of vertebrates and invertebrates as a key stone.⁹

To the best of our knowledge, this is the first study reporting the *Salmonella* contamination (36%) of wild turtles in Iran.

There are many similar studies in other countries with different results. Totally, documented results from similar surveys have publicized that the *Salmonella* contamination in pet turtles fluctuated from 0% to 72.2%¹²⁻¹⁴ and also in free-living turtles from 0% to 15.4%.¹²

Previous studies on the *Salmonella* contamination of tortoises conducted worldwide showed heterogeneous results with the frequency of 100% in *Testudo graeca* tortoises in Morocco, 36.8% in *T. graeca* from North African countries, and 49.1% in tortoises farmed in southern Italy.^{13,15} Frequency of *Salmonella* contamination in sampled Caspian pond turtles is similar to the results stated by Percipalle et al and Savage and Baker, and who detected a prevalence of 38.0% and 34.1%, respectively in tortoises.^{15,16}

In the study of Hidalgo-Vila et al on free-living Valencian turtles, the prevalence of *Salmonella* was 11.0 ± 2.3%.^{17,18}

Table 1. Antibiotic Resistance Pattern of Detected *Salmonella* According to Antibiogram test

Antibiotic	1 ^a	2 ^b	3 ^c
Cotrimoxazole	2 (3.7%)	2 (3.7%)	50 (92.5%)
Nalidixic Acid	4 (7.4%)	1 (1.8%)	49 (90.7%)
Trimethoprim	0 (0%)	1 (1.8%)	53 (98.1%)
Ampicillin	20 (37%)	1 (1.8%)	33 (61.1%)
Tetracycline	18 (33.3%)	2 (3.7%)	34 (62.9%)
Streptomycin	0 (0%)	5 (9.2%)	49 (90.7%)
Gentamicin	0 (0%)	0 (0%)	54 (100%)
Ciprofloxacin	3 (5.5%)	0 (0%)	51 (94.4%)

^a Number and percent of resistant *Salmonella* spp.

^b Number and percent of semi sensitive *Salmonella* spp.

^c Number and percent of sensitive *Salmonella* spp.

The detected frequency in our study was higher than that described in other areas, mainly in Italy (24%),¹⁸ Korea (30% including turtles and other reptiles),¹⁹ Spain (5%),¹³ and the United States (0%).²⁰

Therefore, in comparison to other studies, 38.8% of frequency of *Salmonella* contamination in sampled Caspian pond turtles was related to moderate to high degree *Salmonella* contamination in turtles' population worldwide. This shows that these species can be the main reservoirs of these zoonotic *Salmonella* spp. in the wild and rural ecosystem of Golestan and Mazandaran provinces.

One explanation for high *Salmonella* detection in the sampled turtles can be high survival rates of *Salmonella* in aquatic environments and its frequent isolation from water sources.^{21,22}

However, the main explanations for such differences in detection rate are not clear. Difference in the applied procedures of isolation, species, season of sampling, and geographical conditions can be some clarifying factors for these variable results.^{23,24}

Salmonella typhimurium together with *S. enteritidis* that were detected in the sampled turtles in high value, are highly pathogenic serotypes and are commonly associated to severe infection in humans²⁵; although, the other 3 serotypes, *S. enteric* subsp. *salamae*, *S. enterica* subsp. *arizona*, and *S. enteric* subsp. *houtenae*, which consist less than 9.2%, 9.2%, and 7.4% of the examined pathogens, respectively, are zoonotic pathogens. *S. enteric* subsp. *houtenae* was recently introduced as the cause of meningitis in one child.^{26,27}

It should be mentioned that all 5 detected serotypes in Caspian pond turtles have been documented in other reptiles worldwide, however there is no data about *Salmonella* serotypes in wild turtles of Iran for comparison.

The *Salmonella* serotypes obtained in research of Piasecki et al on reptiles in Poland were similar to the current study.²⁸ Another survey conducted in 1999 in Poland which incorporated over 300 000 animals and food samples showed that the most common serovar of *Salmonella* was *S. enteritidis*.²⁹

Salmonella enterica subsp. *enterica* that was detected with 35.1% frequency in Caspian pond turtles, was found by other investigators to be predominant serotype, too.^{15,28,29}

As difference in the prevalence of *Salmonella* colonization can be seen in animals across different geographical locations, serovars identified in the present study could not be reasonably compared with data from other countries. It seems that serotype dominance depends on different factors such as the region and whole reservoir of *Salmonella* in each ecosystem.

In this study like some other studies, none of the *Salmonella* positive turtles carried more than one serotype.¹³ However, in some studies, one individual of reptiles was detected with simultaneous contamination

with several serotypes. It could have been occurred due to the poor hygienic situations of reptile habitat; a fact that could weaken the immune system and rise the danger of *Salmonella* spread among individuals.³⁰

It is not possible to identify the source of *Salmonella* contamination in the sampled Caspian pond turtles. Nevertheless, regarding the range of Caspian pond turtles, the sources were supposed to be around the villages where the turtles were captured. Therefore, the results of current study revealed the *Salmonella* contamination of wild and rural ecosystems of Golestan province.

As none of the sampled Caspian pond turtles showed clinical sign of salmonellosis, the detected serovars in the Caspian pond turtles can be considered as part of their normal bacterial intestinal flora.

The zoonotic risk for people who live in villages may be emphasized by the fact that the major serotypes detected in the sampled Caspian pond turtles belong to the *S. typhimurium* and *S. enteric* subsp. *enterica*. Accordingly, people who are in close interaction with turtles and their feces are in danger of *Salmonella* contamination.

Obtained results recommend that fundamental principles of hygiene should be applied for humans who are in close contact with Caspian pond turtles in surveyed provinces. Additionally, people should be educated about the *Salmonella* contamination which may occur via Caspian pond turtles. Further studies on the epidemiology, molecular characterization, and pathogenesis of *Salmonella* are needed to assess the actual impact of these organisms on Caspian pond turtles.

Authors' Contributions

EG and SRH developed the original idea. SN and HRE obtained the samples, analyzed the samples, and wrote the manuscript.

Ethical Approval

All procedures performed in this study were in accordance with the ethical standards of the national research committee.

Conflict of Interest Disclosures

The authors have declared that no conflict of interests exists.

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