Prevalence and antimicrobial susceptibility of *Salmonella* in European pond turtles (*Emys orbicularis*) from Gorgan, Golestan Province

Namroodi S.[1*]; Estaji H.[2]

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Abstract

Salmonella spp., the causative agents of salmonellosis, can cause severe illness in immunocompromised warm-blooded animals. European pond turtles (*Emys orbicularis*), common in freshwater habitats of rural Golestan Province, Iran, may act as asymptomatic reservoirs of *Salmonella*. Fecal samples from 105 turtles were collected using sterile swabs for detection of *Salmonella* via conventional culture and PCR. Positive samples underwent serotyping, and antimicrobial susceptibility testing was performed against 14 commonly used antibiotics in human and veterinary medicine. The overall prevalence of Salmonella was 16.6%, with females showing a higher infection rate (18%) than males (14.5%). Juvenile turtles (<1 year) exhibited the highest prevalence. Seasonal variation was significant, with increased infections in summer versus spring. Identified serotypes included S. Typhimurium (41.1%), S. Enteritidis (29.4%), S. Dublin (11.7%), *S. Arizonae* (5.8%), and *S. Houtenae* (5.8%). Antimicrobial resistance varied by serotype, showing highest resistance to enrofloxacin and lowest to gentamicin. The presence of zoonotic *Salmonella serotypes* highlights the turtles' role in pathogen persistence and transmission in the region. Public health interventions, including hygiene education for turtle handling, proper husbandry, and safe water and food practices, are essential to mitigate zoonotic risk, especially for children.

Keywords: *Salmonella*, European pond turtle, transmission, Golestan, Iran

1-Corresponding author: Department of Environmental sciences, Faculty of fisheries and environmental Sciences, Gorgan University of Agricultural Sciences & Natural Resources, Gorgan, Iran.
2-Department of Pathobiology, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.
*Corresponding author's Email: namroodi@gau.ac.ir

Introduction

The genus *Salmonella* belongs to the family *Enterobacteriaceae*, a group of facultative anaerobic, motile, Gram-negative bacteria. These organisms show significant environmental persistence, persisting for several weeks in dry environments and up to ten months in wet environments. *Salmonella* is taxonomically divided into two species: *Salmonella bongori* and *Salmonella enterica* with typhoidal and non-typhoidal serovars. To date, approximately 60 serogroups and 2,600 serotypes of *Salmonella* have been recognized in both warm- and cold-blooded animals. Worryingly, over 2,000 serotypes hold zoonotic potential and have been detected in human infections (Jones *et al*., 2025; Scheelings *et al*., 2011**;** Smith *et al*., 2025).

Interestingly, *Salmonella* presence in the gastrointestinal tract of asymptomatic hosts does not automatically lead to disease. However, in animals experiencing immunosuppression due to conditions such as pregnancy, infection with highly virulent pathogens, or the use of anticancer drugs, *Salmonella* may disseminate from the intestinal vesseles to other organs, including bone marrow, central nervous system (brain) and eyes. In such cases, clinical signs extend beyond gastrointestinal disturbances (e.g., diarrhea, vomiting, abdominal cramping), potentially leading to septicemia, seizures, blindness and, if left untreated, death (Bergmire-Sweat *et al*., 2008; MacDonald *et al*., 2019).

While most cases of *Salmonella*-associated infection (salmonellosis) are self-limiting gastrointestinal infections, the unselective use of antibiotics—particularly in industrial livestock and poultry farming—has led to the appearance of antimicrobial-resistant strains. This phenomenon confuses treatment approaches in both human and animals. So, survey on antimicrobial resistance patterns of *Salmonella* isolates from animal reservoirs is critical for disease management (Aljasir *et al*., 2025; Chea *et al*., 2025; Nagpala *et al*., 2025; Scheelings *et al*., 2011).

It is estimated that about 90% of reptiles—including snakes, lizards and turtles—harbor *Salmonella* asymptomatically in their gastrointestinal tracts. *Salmonella* transmission among turtle populations occurs through multiple routes, including maternal transmission to eggs, mating, contact with contaminated environments, exposure to infected insects and consumption of contaminated food and water (Mermin *et al*., 2004; Richards *et al*., 2004; Tomastikova *et al*., 2017; Pees *et al*., 2023; Clancy *et al*., 2016; Sting *et al*., 2013; Schröter *et al*., 2006).

Studies conducted in various regions worldwide indicate that wild aquatic turtles (both freshwater and marine) exhibit lower *Salmonella* prevalence compared to other reptiles. However, periodic fecal shedding of zoonotic *Salmonella* serotypes has been reported in multiple turtle species. Some documented serotypes include *S. Poona*, *S. Pomona*, *S. Marina*, *S. Stanley*, *S.*

Abony, *S. Lichfield*, *S. Enteritidis*, *S. Sandiego*, *S. Agbeni*, *S. Montevideo*, *S. Houtenae* and *S. Newport*, with reported prevalence rates ranging from 0% to 15% (Pees *et al*., 2023; Bergmire-Sweat *et al*., 2008; Mermin *et al*., 2004; Nagano *et al*., 2006; MacDonald *et al*., 2019; Walters *et al*., 2016; Basler *et al*., 2015)

Freshwater turtles, such as the European pond turtle (*Emys orbicularis*), exhibit biological and ecological behaviors that increase their interaction with human populations, particularly in rural areas (De Jong *et al*., 2005; Angulo *et al*., 2010).

The European pond turtle is one of 50 recognized freshwater turtle species worldwide. In Iran, this species is distributed from the northeast to the northwest. These turtles inhabit a variety of aquatic environments, including rivers, lakes, small ponds and vegetated wetlands. They can travel up to one kilometer in search of food. Juvenile turtles are predominantly carnivorous, feeding on arthropods, snails, amphibian larvae and small fish, while adults exhibit increasing herbivorous tendencies (Ficetola and De Bernardi, 2006; Fritz and Chiari, 2013).

Mating of adult individuals begins in spring and a female may mate multiple times and lay eggs several times per year, with each clutch containing between 3 and 15 eggs (Hosseinian Yousefkhani *et al*., 2022).

Golestan Province, located in northeastern Iran, is characterized by a predominantly temperate and humid climate. The region supports diverse wildlife, including susceptible mammalian species such as the Turkmen fox (*Vulpes vulpes turkmenica*) and the Persian leopard (*Panthera pardus saxicolor*). The Province's fertile agricultural lands have also contributed to the development of numerous rural settlements, increasing human-wildlife interactions and the potential for zoonotic disease transmission (Yelghei *et al*., 2020).

Due to the presence of European pond turtles in stagnant freshwater bodies near rural and wild regions, obtaining data on *Salmonella* epidemiology (dominant serotypes and antimicrobial resistance patterns) in this species is crucial. This study aims to fill this knowledge gap by assessing the prevalence of *Salmonella* in European pond turtles, characterizing the isolated serotypes and determining their antibiotic resistance profiles. The findings of this research will contribute to more effective disease management strategies for salmonellosis in freshwater turtle populations, rural populations and native wildlife.

Materials and methods

Sampling

European pond turtles were collected from rivers and water bodies in the vicinity of the villages of Toshkan, Shast Kola, Valaghuz, Chahardeh, Shamushak, Ilvar and Nodeh Malek near Gorgan City. Data on age, sex, sampling time and location were recorded.

Salmonella detection

The detection of *Salmonella* was conducted using fecal swabs obtained from 105 European pond turtles (67

males and 42 females), ranging in age from 1 to 40 years, during the spring and summer seasons, following the methodology described by Musilín *et al*., (2025). After sample collection, all turtles were released back into their natural habitats. Fecal swabs were initially inoculated into Selenite F enrichment broth and incubated at 37°C for 24 hours. Subsequently, an aliquot of the enriched sample was streaked onto MacConkey agar and incubated for an additional 24 hours at 37°C. Colonies appearing colorless on MacConkey agar were further subcultured onto Salmonella-Shigella agar (SSA). Presumptive *Salmonella* colonies on SSA were subjected to biochemical characterization, including urease, peptone water, Simmon's citrate, methyl red (MR) and Voges-Proskauer (VP) tests, to confirm the presence of *Salmonella spp*. (Zahraei Salehi *et al*., 2010) . Also, For molecular detection of *Salmonella* isolates, the universal gene primers: ST 11: 5'-GCCAACCATTGCTAAATTGGCGC A-3' and ST 15: 5'-GGTAGAAATTCCCAGCGGGTACT GC-3' were used (Soumet *et al*., 1999). For serotyping, a portion of a confirmed *Salmonella*-positive colony from triple sugar iron (TSI) agar was suspended in 85% saline and a drop of the prepared suspension was placed onto a glass slide. The agglutination reactions were observed under a microscope following the sequential addition of specific *Salmonella* antisera targeting O antigens (B, C, D and E) and H antigen. A control slide containing only normal saline without antisera was included to ensure the validity of the test results (Issenhuth-Jeanjean *et al*., 2014). To assess antimicrobial susceptibility, the *Salmonella* isolates were inoculated onto Mueller-Hinton agar and antibiotic susceptibility testing was performed using the disk diffusion method with 15 commonly administered antibiotics in veterinary and human medicine. The inhibition zone diameters were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines, classifying the isolates as susceptible, intermediate, or resistant based on the standardized criteria (Humphries *et al*., 2018). The associations between independent variables, including age, sampling season and sex, with the prevalence and antimicrobial resistance patterns of *Salmonella* isolates were analyzed using SPSS software version 26.,T-test and chi-square test, with a p-value of <0.05 considered indicative of a significant association.

Results

Salmonella contamination was observed in 17 out of 105 turtles (16.6%). The prevalence of infection in female turtles (18%) was significantly higher than in male turtles (14.5%). The highest rate of Salmonella contamination was detected in turtles under one year of age (25%). The number of infected turtles in the summer (20%) was significantly higher than in the spring (9%). The identified subspecies of *Salmonella* included *Typhimurium*, *Enteritidis*, *Dublin*, *Arizona* and *Houtenae*, with frequencies

of 41.1%, 29.4%, 11.7%, 5.8% and 5.1%, respectively. The antibiotic resistance patterns varied among different serotypes, with the highest and lowest resistance observed against Enrofloxacin and Gentamicin, respectively (Table 1).

Table 1: Antibiotic resistance patterns of isolated *Salmonella* strains from European pond turtles (*Emys orbicularis*).

Antibiotic	Resistant (n, %)	Intermediate (n, %)	Susceptible (n, %)
Enrofloxacin	7 (41%)	4 (23%)	6 (35%)
Colistin	4 (23.5%)	6 (35%)	7 (41%)
Erythromycin	1 (5.8%)	5 (29.4%)	11 (64.7%)
Trimethoprim	4 (23.5%)	1 (5.8%)	12 (70.5%)
Doxycycline	3 (17.6%)	6 (35%)	9 (52.9%)
Florfenicol	2 (11.7%)	5 (29.4%)	10 (71.4%)
Lincomycin	1 (5.8%)	3 (17.6%)	13 (76.4%)
Tetracycline	1 (5.8%)	4 (23.5%)	12 (70.5%)
Nalidixic Acid	0 (0%)	3 (17.6%)	14 (82.3%)
Streptomycin	0 (0%)	4 (23.5%)	13 (76.4%)
Ampicillin	1 (5.8%)	4 (23.5%)	12 (70.5%)
Gentamicin	0 (0%)	2 (11.7%)	15 (88.2%)
Neomycin	2 (11.7%)	5 (29.4%)	10 (71.4%)
Furazolidone	0 (0%)	4 (23.5%)	13 (76.4%)
Ciprofloxacin	0 (0%)	5 (29.4%)	12 (70.5%)

Discussion

The single fecal swab collection method used in this study likely resulted in an underestimation of the true *Salmonella* prevalence, as shedding is intermittent in reptiles (Nowakiewicz *et al*., 2015). Previous research has reported *Salmonella* prevalence in wild turtle populations ranging from 0% to 15% (Pees *et al*., 2023). Contamination of European pond turtles with *Salmonella* has also been reported in other parts of the world. In three different studies conducted in Spain, *Salmonella* contamination rates of 11%, 8% and 15.4% were reported in European pond turtles (Hidalgo-Vila *et al*., 2007: Hidalgo-Vila *et al*., 2008: Marin *et al*., 2013). In Poland, Latvia and Italy, *Salmonella* contamination rates of 11.7%, 0% and 0% have been reported in European pond turtles, respectively (Zuffi *et al*., 2007; Nowakiewicz *et al*., 2015; Umbraško *et al*., 2023). The difference between results of similar studies with current study can be influenced by various factors, including hygiene, humidity, temperature, population density, diet and species. Carnivorous turtles generally exhibit higher *Salmonella* shedding rates than herbivorous species. Temperature plays a crucial role in bacterial proliferation, survival and host infection rates (Scheelings *et al*., 2011, Pees *et al*., 2023). Pasmans *et al*., (2002) have shown that raising the body temperature of red-eared sliders to 37°C, similar to mammalian body temperatures, significantly increases *Salmonella* proliferation and shedding (Pasmans *et al*., 2002).

In a study by Namroodi *et al*. (2017) on *Mauremys caspica* (Caspian pond turtle) in Golestan Province, a markedly higher contamination rate (38.8%) was reported (Namroodi *et al*., 2017a). The discrepancy in contamination rates between *E. orbicularis* and *M. caspica* highlights the influence of species-specific factors and dietary habits on prevalence outcomes.

Studies on rural cats, rural dogs, and brown rats in temperate and humid rural areas of Golestan Province have reported contamination rates of 14.7%, 19.4%, 10%, 16.6% and 13.1%, respectively (Namroodi and Behine, 2016; Namroodi *et al*., 2016a; Namroodi *et al*., 2016b; Namroodi, 2019). Detection of 16.6% *Salmonella* contamination in European pond turtles seems similar with other animal species around Gorgan City and it can be due to consumption of similar contaminated food and water soureces (Pees *et al*., 2023).

The identified serotypes showed difference by region and species among turtles, in similar studies. A systematic review of studies on reptiles found that three subspecies, *Enterica*, *Salamae* and *Diarizonae*, were the most commonly serotypes with 70.3%, 29.7% and 19.6% frequency, respectively. However, some reptiles exhibited none of these subspecies (Pees *et al*., 2023).The results of the present study partially align with previous studies on reptiles. Researches on European pond turtles in Spain and Poland identified contamination with *Salmonella* subspecies *Potsdam*, *Salamae*, *Thompson*, *Diarizonae*, *Typhimurium*, *Houtenae and Newport* (Hidalgo-Vila *et al*., 2007; Hidalgo-Vila *et al*., 2008; Marin *et al*., 2013; Nowakiewicz *et al*., 2015). The isolation of Salmonella *Dublin* in the present study may represent the first documented case in this species worldwide.

Previous studies on animals in Golestan Province, including rural dogs, brown rats and rural cats, identified *S. Enteritidis* and *S. Typhimurium* as prevalent strains, with subspecies *Dublin* and *Arizona* isolated from rural dogs and jackals, respectively (Namroodi *et al*., 2016a, Namroodi *et al*., 2016b, Namroodi, 2019). Similarly, S. *Typhimurium* and the subspecies *Salamae*, *Arizona*, *Enterica* and *Houtenae* were identified in Caspian pond turtles in Golestan Province (Namroodi *et al*., 2017a). These findings suggest close ecological interactions between animal species and the use of shared water and food resources in the studied region. Although the prevalence of *Salmonella* contamination was higher in female turtles than in males, the difference was not statistically significant. Similar findings were reported by Namroodi *et al*. (2017) for Caspian Pond turtles in rural Golestan Province) Namroodi *et al*., 2017a). Also, Hernandez *et al*., (2021) observed significantly higher contamination in female turtles across six aquatic turtle species while Sringam *et al*., (2021) found significantly higher contamination in male freshwater turtles in Thailand (Hernandez *et al*., 2021; Sringam *et al*., 2021). These differences

may be because of sex-based variations in dietary habits, difference in mating behavior and predation tendencies among turtle species (Roques *et al*., 2004; Casalino *et al*., 2021)

Increased *Salmonella* contamination in juvenile turtles, as observed in the present study and others, is commonly attributed to weaker immune systems in younger individuals (Sringam *et al*., 2021). Additionally, the carnivorous diet preference of young pond turtles compared to the omnivorous or herbivorous tendencies of adults may influence contamination rates (Ficetola and De Bernardi, 2006). However, Nowakiewicz *et al*., (2015) found no *Salmonella* contamination among 96 juvenile European pond turtles in Poland.

In this study, the number of infected turtles in the first weeks of summer was higher compared to the spring. Given the moderate cimate condition and abundant rainfall in beginning of summer in 1403 (2024), along with favorable conditions for the growth of *Salmonella* in the environment and the body of the European pond turtle—a cold-blooded species—this outcome was expected. Due to limited access to individuals of this species during the autumn and summer months, sampling was not conducted in these two seasons. Considering the favorable climatic conditions of first weeks of summer in 2024 and the stress induced by mating and egg-laying (which reduce the immune defense and result in higher *Salmonella* excretion in the intestines) occurring early in the summer, higher *Salmonella* contamination in the summer weeks, as compared to spring, is plausible (Hailey and Willemsen, 2000; Pasmans *et al*., 2002; Hernandez *et al*., 2021).

In most studies conducted on animals such as Caspian Pond turtles in Golestan Province and European pond turtles in Spain, only a single subspecies or species of *Salmonella* has been isolated from each animal (Hidalgo-Vila *et al*., 2007; Marin *et al*., 2013). However, the present study observed *Salmonella* infection involving more than one subspecies or species in the infected turtles. This finding appears to be significantly influenced by the level of contamination, the environmental disturbances affecting the species under study and potentially the immune status of studies turtles (Pees *et al*., 2023). Previous studies have reported resistance to antibiotics such as Erythromycin, Sulfisoxazole, Gentamicin, Amoxicillin and Ampicillin in turtle populations from regions where these antibiotics are commonly used (Ebani *et al*., 2005). Since the pattern of antibiotic resistance in *Salmonella* strains isolated from animals and turtles is entirely dependent on the specific antibiotics typically used in the region, it is not meaningful to compare the antibiotic resistance patterns of *Salmonella* in turtle populations across different parts of the world (Ebani *et al*., 2005). In study by Namroodi *et al*., (2017) on antibiotic resistance patterns in *Salmonella* strains isolated from Caspian Pond turtles in Golestan Province-areas that overlap with those of

European pond turtles—the highest resistance was observed against Ampicillin (37%). In contrast, other studies conducted in Golestan Province on dog populations (Streptomycin), cats (Streptomycin), rats (Streptomycin) have reported a different antibiotic resistance pattern compared to the current study, where the highest resistance was found against Enrofloxacin (Namroodi *et al*., 2016a, Namroodi *et al*., 2016b, Namroodi *et al*., 2017b; Namroodi, 2019). Considering that previous studies in Golestan Province were conducted between 2015 and 2018 and the time gap between those studies and the present one, it is likely that the pattern of antibiotic usage in the province has changed in recent years. This is because antibiotic resistance patterns are typically transmitted via plasmids and the highest resistance is often observed against the most commonly used antibiotics in the region (Ebani *et al*., 2005).

Conclusion

The serotypes isolated from the European pond turtles in this study reflect the serotypes present in the local region, as well as in the gastrointestinal tracts of the local animal fauna (including birds, reptiles, domesticated and wild mammals and humans). This is because runoff entering the areas where European pond turtles reside becomes contaminated through contact with the feces of these animals, ultimately polluting the turtles' environment. Additionally, since all of the serotypes identified in this study have the potential to cause salmonellosis in humans and wildlife, the risk of the antibiotic-resistant *Salmonella* transmission to the native wildlife and human population in the studied villages seems inevitable. Therefore, it is essential to implement preventive measures to limit the transmission of *Salmonella* from European pond turtles to the local population, particularly children, through educational initiatives regarding proper hygiene when interacting with these animals, water hygiene and food safety.

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Conflict of interest

No conflict of interest has been declared by the authors.

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Ethical considerations

All experimental procedures involving animals (fecal sampling) were approved by the Animal Welfare and Ethics Committee of Gorgan University Agricultural Science and Natural resources, Gorgan, Iran (approval number: N.T166849).

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