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Research Article

Salmonella detection in Brown Rats (*Rattus norvegicus*) and Seasonal Variations: a case study in Gorgan, North-East of Iran

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ABSTRACT

Salmonella is a Gram-negative bacterium that causes disease in both warm- and ld-blooded animals, exhibiting sensitivity to dryness and high temperature. Urban dents act as asymptomatic reservoirs of *Salmonella*, posing a significant public health ;k, especially to children and immunocompromised individuals. Gorgan, the capital of olestan Province in northeastern Iran, is characterized by a dense population of brown ts. The city was divided into 20 districts, and five rats from each district were stematically captured using handmade traps during all four seasons. Fecal samples ere analyzed using both conventional culture techniques and molecular diagnostic says. *Salmonella* was detected in 24% of the sampled rats (24 out of 100). Two nically relevant serotypes—*S. Typhimurium* (n=17) and *S. Enteritidis* (n=7)—were entified. No statistically significant association was observed between infection rates d the rodents' age or sex. A distinct seasonal trend in prevalence was observed, with ntamination rates of 36% in spring, 28% in summer, 20% in autumn, and 12% in nter. The tow detected serotypes frequently implicated in human salmonellosis. ven the zoonotic potential of *Salmonella*, these findings emphasize the need for inimizing the risk of bacterial transmission from rodents to humans, improving urban iblic health and enhanced urban health measures, including effective waste disposal, odernization of sewage systems, and robust rodent control programs. The observed asonal fluctuation emphasizes the need for year-round monitoring and targeted terventions during high-risk periods for application of better disease management ategies.

1. Introduction

Rodents are among the most numerous mammalian species on Earth, primarily due to their high reproductive capacity. Among the various species within this family, the brown rat (*Rattus norvegicus*), commonly referred to as the sewer rat or Norway rat, is one of the most prevalent in urban environments. These omnivorous rodents primarily feed on human food waste—such as bread, grains, fruits, vegetables, and meat. In cases of food scarcity, they are also known to consume materials found in municipal sewage systems (Hazratian, Naderi and Mollashahi 2017). They also serve as important reservoirs of zoonotic pathogens, such

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as *Salmonella* spp., which pose significant risks to public health in densely populated areas. *Salmonella* is a genus of motile, rod-shaped, facultatively anaerobic, Gram-negative bacteria, with two genera belonging to the family *Enterobacteriaceae*. These organisms are capable of surviving in a wide range of environments,, including the gastrointestinal tracts of animals and humans, as well as water, soil, and various food sources. The survival of these bacteria is heavily influenced by environmental factors such as temperature and moisture, with variations in different climate types. *Salmonella enterica*, which is subdivided into six subspecies, includes the majority of serovars responsible for infections in humans and warm-blooded animals. In contrast, *Salmonella bongori* is predominantly associated with cold-blooded animals (Ibarra-Cerdeña et al. 2024).

Salmonella can cause a spectrum of diseases in humans, most notably gastroenteritis and typhoid fever. Gastroenteritis, the most common clinical manifestation, is characterized by diarrhea, abdominal cramps, vomiting, and fever. Typhoid fever, a more severe systemic illness, typically presents with prolonged high fever, weakness, rash, and intestinal complications; if left untreated, it can result in life-threatening outcomes. In addition, systemic salmonellosis may lead to bacteremia and infection of internal organs (Ajmera and Shabbir 2024). *Salmonella* infection, particularly in young children and the elderly, can lead to severe complications and, in some cases, death (Stanaway et al. 2019).

Salmonella is primarily transmitted through the consumption of water and food contaminated with the bacteria. Preventive measures focus on good personal hygiene, sanitation practices, and ensuring that food is properly handled and thoroughly cooked (Ajmera and Shabbir 2024).

Globally, zoonotic diseases transmitted by urban rodents have been increasing, with *Salmonella*, *Leptospira*, and *Yersinia pestis* frequently implicated in disease outbreaks. Rapid urbanization, inadequate waste disposal systems, and increasing contact between humans and rodents have intensified this public health concern. Consequently, urban rodents are now widely recognized as significant contributors to the global burden of zoonotic infections (Akhtar et al. 2023).

Gorgan, the capital of Golestan Province, is located in northeastern Iran and has a population of approximately 500,000. The Gorgan River flows through the city, which is characterized by a temperate and pleasant climate (Meftahhalaghi and Ghorbani 2015). Such environmental conditions are conducive to both the proliferation of brown rats and the persistence of *Salmonella* in the urban ecosystem.

Despite numerous global studies, there is a lack of localized data on the prevalence, seasonal trends, and serotype diversity of *Salmonella* in urban rodents in Iran. This study seeks to address that gap. The novelty of this research lies in its examination of seasonal variation in *Salmonella* prevalence among urban brown rats in Gorgan, Iran, using both culture-based and molecular detection methods. By identifying circulating serotypes and their seasonal distribution, this study provides critical information for urban health authorities to develop targeted strategies for disease surveillance and rodent control.

2. Materials and Methods

This descriptive cross-sectional study was conducted in the city of Gorgan, northeastern Iran. The city was divided into 20 distinct urban districts, and a total of 100 brown rats (*Rattus norvegicus*) were trapped (5 rats per area). All trapping and handling procedures were conducted in accordance with the Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education (Sikes et al., 2016), ensuring non-lethal capture and safe release of the animals into their natural environment after sampling. All experimental protocols were approved by the Ethical Committee of Gorgan University of Agricultural Sciences and Natural Resources, and efforts were made to minimize animal suffering and ensure humane handling.

The sampling was conducted across four seasons to assess potential seasonal variation. The rats were captured using handmade baited traps. To prevent stress and avoid contamination, each captured rat was placed in a clean, individually disinfected container immediately after capture. All handling procedures were carried out using disposable gloves and sterilized tools to eliminate the risk of cross-contamination. After sampling, each rat was released at the site of capture to comply with ethical and wildlife protection guidelines.

Fresh fecal samples were collected and immediately inoculated into Selenite F broth for selective enrichment. The samples were then streaked onto selective and differential agar media, including MacConkey, SS, and XLD agar, and incubated at 37°C for 24–36 hours. Suspected *Salmonella* colonies based on morphology were selected and subcultured onto biochemical media including TSI, peptone water, Simmon's citrate, urea, and MR-VP to confirm identity (Lee et al. 2015).

To determine the serotype, a sample was first taken from a positive *Salmonella* colony grown on TSI culture medium and diluted in 85% saline solution. A drop of the prepared solution was placed onto a slide, and the reactions were observed under a microscope while adding antisera. A control slide, on which only normal saline was placed and antisera were added, was also included for comparison (Issenhuth-Jeanjean et al. 2014).

To confirm *Salmonella* at the molecular level, the invA gene—recognized for its specificity and conservation among *Salmonella* spp. was targeted for PCR amplification. Primers (Forward: 5'-ACA GTG CTC GTT TAC GAC CTG AAT-3', Reverse: 5'-AGA CGA CTG GTA CTG ATC GAT AAT-3'), which generate a 245 base pair fragment used in this study were adopted from the method developed by Arora and Singh 2023 (Arora, Bithel and Singh 2023).

PCR was performed with the following thermal cycling conditions: initial denaturation at 95°C for 5 minutes; 30–35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 7 minutes. Amplified products (245 bp) were visualized by electrophoresis on 1.5% agarose gel using 1X TBE buffer, run at 80 V and 30 mA for 75 minutes, and examined using a gel documentation system. The *invA* PCR assay using the primer set described above has demonstrated high inclusivity and exclusivity in validation studies. Specifically, it correctly identified 99.6% of *Salmonella* strains tested and showed no cross-reactivity with non-*Salmonella* strains, confirming its high specificity. The detection limit of the *invA* PCR assay has been reported to be as low as 10 genomic copies per reaction, corresponding to approximately 5–50 CFU, depending on the sample matrix and DNA extraction method (Awang et al. 2021). Statistical analysis was performed using SPSS software, version 26. The Chi-square test and Anowa one-way test were used to evaluate the association between gender/sex and *seasons*. A p-value of ≤ 0.05 was considered statistically significant.

3. Results

Salmonella bacteria were isolated from 24 (24%) of 100 fecal samples using both culture and molecular methods (Table 1, Figure 1). Previous studies conducted in Golestan Province have investigated Salmonella contamination levels in fecal samples from rural and wild animals within the ecosystem surrounding Gorgan. Contamination rates ranging from 10% to 36% have been reported in various species, including Caspian pond turtles (36%), rural cats (14.7%), rural dogs (19.4%), jackals (16.6%), and sheep (10%) (Namroodi and Behine 2016, Namroodi, Estaji and Dehmordeh 2016a, Namroodi et al. 2017a, Namroodi, Staji and Mazandarani 2016b, Namroodi, Staji and Sharafi 2017b). Given that other sampling environments in Golestan Province differ from the current study in terms of human and animal density, as well as land use, a comparison between the results of the previous studies and the present study is not feasible. However, the recent study demonstrates that *Salmonella* contamination in urban areas of Golestan Province is as prevalent as in rural areas. Henzler et al. (1992) reported a global average prevalence of *Salmonella* infection in rodents ranging from 0% to 15% (Henzler and Opitz 1992).

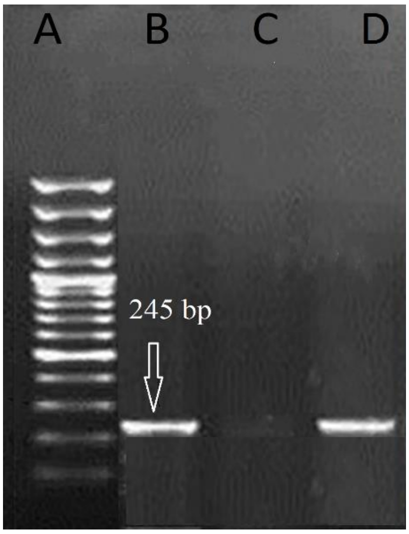

Fig 1: A: DNA ladder, B: positive sample, C: negative control, D: positive control

Table 1. Biochemical test results for 24 *Salmonella* isolates**.** These biochemical profiles are consistent with the characteristics of *Salmonella* serotypes *S. Typhimurium* and *S. Enteritidis* .

Biochemical Test	Result	Interpretation
Triple Sugar Iron (TSI) Agar	Red slant / Yellow butt, H_2S positive, Gas positive	Glucose fermentation with H_2S and gas production
Simmon's Citrate	Positive (color change from green to blue)	Ability to utilize citrate as sole carbon source
Urease	Negative (no color change)	Absence of urease enzyme production
Methyl Red (MR)	Positive (red color development)	Stable acid production from glucose fermentation
Voges-Proskauer (VP)	Negative (no color change)	Absence of acetoin production from glucose fermentation

4. Discussion

In Iran, Hadadian et al. conducted a study on the feces of 290 rodents from poultry farms around Tehran to assess *Salmonella* contamination, reporting 28 positive (9.65%) samples within the study population (Hadadian et al. 2012).

Comparing current results, Namroodi (2019) reported lower (15%) *Salmonella* contamination rate of rural brown rats in villages of Golestan Province with *Enterica* and *Typhimurium* serotypes (Namroodi 2019). The use of human food sources and the proximity to human settlements may have contributed to the higher contamination levels of urban rats of this study compared to rural rats. As, in the absence of access to human food leftovers in urban areas, rats may increasingly rely on sewer systems for food, which also elevates the likelihood of contact with human feces, a significant source of *Salmonella* transmission.

Hilton et al. (2002) reported a *Salmonella* contamination rate of 7% in brown rats in the West Midlands region of England (Hilton, Willis and Hickie 2002) . Himsworth et al. (2015) reported a *Salmonella* contamination rate of 0.5% in fecal samples from house and brown rats in Vancouver, Canada (Himsworth et al. 2015).

Ribas et al. (2016) investigated the *Salmonella* contamination rate in fecal samples from brown and Polynesian rat populations in urban areas of Thailand and reported that 49.1% of the samples were contaminated (Ribas et al. 2016).

However, Burt et al. (2018) reported a 0% *Salmonella* contamination rate in house mice sampled in Utrecht, the Netherlands (Burt et al. 2018).

Falay et al. (2022) reported an 8% *Salmonella* contamination rate in fecal samples from house and brown rat populations in urban areas of the Congo. Genetic analyses revealed a high degree of similarity between the *Salmonella* strains isolated from rats and those isolated from humans in the same region (Falay et al. 2022). Shah et al, (2023) and Niu et al., (2023) analyzed intestinal mircobium of *Rattus norvegicus* in Pakistan and China , respectively (Shah et al. 2023, Niu et al. 2023). They reported presence of wide range of zoonotic bacteria in intestine of sampled rats except *Salmonella*.

Rodent species have also been shown to influence the prevalence of *Salmonella* infection. For instance, in a study on 100 rodent from differrents farms of Egypt, *Rattus norvegicus* typically exhibits a higher rate of *Salmonella* carriage compared to *Mus musculus* (Nashwa and Ebtesam 2007). Various other factors, such as the climatic conditions of the study area, availability of water resources, methods of human sewage disposal, and land use patterns, can influence the level of *Salmonella* contamination in animals and may account for the differences observed across various studies with current study (Galán-Relaño et al. 2023)

Two serotypes, *S. enteritidis* and *S. typhimurium*, were identified in the studied population, with frequencies of 13% and 11%, respectively. Similar findings have been reported in other studies conducted on animals in Golestan Province, where rural dog populations, jackals, sheep, rural brown rats, and rural cats were also found to be infected with *S. enteritidis* and *S. typhimurium*)Namroodi 2019, Namroodi et al. 2016a, Namroodi et al. 2016b, Namroodi et al. 2017b, Namroodi and Behine 2016). Therefore, it appears that *S. enteritidis* and *S. typhimurium* are among the dominant serotypes circulating within the animal population of Golestan Province. *Salmonella* serotypes isolated from brown rats have also been investigated in various regions worldwide. In Vancouver, Canada, *S. Derby*, *S. Indiana*, and *S. Enteritidis* have been reported. In urban areas of the Congo, *S. Kapemba*, *S. Weltevreden*, *S. Typhimurium*, and *S. Dublin* have been identified. In Thailand, *S. Typhimurium* and *S. Weltevreden* were isolated from brown rats, while in New York, *S. Enterica* has been reported (Falay et al. 2022, Ribas et al. 2016, Himsworth et al. 2015, Firth et al. 2014). *Salmonella* serotypes exhibit varying degrees of host specificity, which influences their distribution across different animal species. While some serotypes are host-restricted, others possess a broader host range and are capable of infecting multiple species, including rodents (Wallis 2006). Genetic and ecological factors play a significant role in shaping the diversity of *Salmonella* serotypes found in rodent populations (Swanson et al. 2007).

The frequency of *Salmonella* infection was similar between males and females. However, in some animal species, infection rates have been reported to differ between sexes. It has been suggested that such differences may be attributed to variations in dietary habits between males and females, which could influence exposure to *Salmonella* (Floch 2015). Studies suggest that gender-based differences in rodent susceptibility to *Salmonella* infection may exist; however, such differences are influenced by various factors, including animal species, environmental conditions, and immune status. Sexual behavior, particularly in male rodents, also plays a role. For instance, exposure to female odor has been shown to elicit physiological responses in male mice, such as elevated testosterone levels. Although this hormonal change does not directly suppress the immune system, it increases the energy demands required to manage the infection, potentially leading to weight loss during the course of illness (Zala, Potts and Penn 2008). Sex-based differences in immune responses have been observed, with female mice exhibiting higher antibody levels compared to males, suggesting a potential disparity in immune system efficiency between genders (Caron et al. 2005).

Salmonella contamination was similar in different age groups of sampled rats in current study. Rodent age significantly influences susceptibility to *Salmonella* infection, with both juvenile and elderly mice demonstrating greater vulnerability compared to adults. This increased susceptibility is largely attributed to age-related changes in the immune system, which compromise the host’s ability to mount an effective response against infection (Ren et al. 2009, Burns-Guydish et al. 2005). Ren et al., 2009 reported that old C57BL/6 mice have significantly higher colonization rates of *Salmonella* in various organs compared to young mice, attributed to impaired immune responses (Ren et al. 2009). Conversely, Burns-Guydish et al., 2005 showed that neonatal and suckling BALB/c mice are more susceptible to *Salmonella* infections at lower inoculum sizes than adults, indicating a different susceptibility pattern across ages (Burns-Guydish et al. 2005). In summary, while age does influence *Salmonella* susceptibility in laboratory settings, different factors (such as species as it mentioned) in wild rodent populations may complicate this relationship, indicating a need for further research into the dynamics of contamination across different rodent ages and habitats.

Salmonella contamination varied meaningfully (p= 0.00) across different seasons as follows: spring= 36%, summer=28%, autumn=20%, winter=12%. Increased temperatures and specific seasonal conditions have been shown to correlate with higher *Salmonella* contamination rates in both environmental and animal reservoirs. Warmer temperatures are associated with increased *Salmonella* notifications, with a twofold increase in risk at temperatures around 33°C compared to average temperatures (Robinson et al. 2022)

Higher *Salmonella* contamination has been reported in hot seasons by Wales et al., 2007. They reported that Rodents can act as vectors for *Salmonella*, and their activity may increase during warmer seasons, potentially elevating contamination rates in agricultural settings (Wales et al. 2007).

The seasonal variation in *Salmonella* contamination underscores the critical importance of monitoring temperature and humidity levels across different regions to inform effective disease management strategies.

Given the zoonotic potential of *Salmonella* and the close interaction between rodents and human environments, particularly in densely populated urban areas, the presence of these pathogens in rodent feces represents a considerable public health risk. So it is crucial to implement preventive measures, including proper waste management, improvements to sewage systems, and control of rat populations in urban areas. These measures can reduce the risk of *Salmonella* transmission from urban rats to humans and play a significant role in enhancing the overall public health of the community.

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