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Study of the prevalence of *Salmonella* Arizonae infection in backyard and commercial turkey flocks in Golestan, Mazandaran, Gilan, and Tehran provinces

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A B S T R A C T

Arizona infection or avian arizonosis (AA) is an acute or chronic egg-transmitted disease of primarily young turkey poults characterized by septicemia, neurological signs, blindness, and increased mortality, caused by caused by the bacterium *Salmonella* enterica subsp. Arizonae (*S*. Arizonae). This study investigated the infection rate of *Salmonella* Arizonae in backyard and commercial turkeys of Golestan, Mazandaran, Gilan, and Tehran provinces**.** Fecal samples taken from industrial and backyard turkeys were transferred to cysteine selenite enrichment medium, incubated at 37° C for 24 hours, and then cultured linearly in selected media of Chromagar, *Salmonella-Shigella*, XLD, and McConkey agar plates and exposed to 37° C for 24 hours. Suspected *Salmonella* colonies were cultured in differentiated TSI and urea medium for confirmation and incubated at 37° C for 24 h. In this study, the microbial culture method was used by pre-enrichment, specific, and differential media to determine the level of contamination of backyard and industrial turkey flocks in four provinces of Golestan, Mazandaran, Gilan, and Tehran**.** All samples were tested for *Salmonella* infection, but positive samples were found for *Salmonella*. Only 22 specimens were infected with *Proteus*. Our information about the Iranian turkey industry is limited compared to worldwide studies. This may be due to Iran's less widespread use of turkey production. The non-isolation of *Salmonella* in this study is inconsistent with some of the few studies in the country, which may be due to differences in the type of sampling, geographical location, and, materials and methods of work. Due to the growth of the turkey industry in the country, the importance of studying *Salmonella* and other pathogens is felt more than ever.

Keywords: *Salmonella Arizonae, Arizonosis, Birds, Turkey, Iran*

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1 Introduction

Despite significant technological advancements and health measures throughout all stages of poultry production, salmonellosis and *Salmonella* infections remain a persistent threat to human and animal health. These infections are widespread in chickens worldwide, and when left uncontrolled, they cause severe economic damages. The economic losses include high mortality rates in poultry during the first weeks of life, high costs of medication, reduced egg production in breeding flocks, poor quality of poultry meat, high expenses for eradication and control measures. Furthermore, the transmission and spread of *Salmonella* occur through both vertical and horizontal routes. However, the most critical aspect is the continuous risk of *Salmonella*-contaminated turkey meat and its meat products to public health. The economic costs of salmonellosis in humans are also substantial, including absenteeism from work, medical treatment, research expenses, control measures, disposal of contaminated materials, and loss of leisure time (1). Consumers' increasing interest in organic chicken production and the high demand for natural and healthier products are evident. Losing consumer trust and confidence in the quality and safety of poultry meat is a significant challenge. Turkey meat can harbor various foodborne pathogens, such as *Salmonella* and *Campylobacter*, which are the most common causes of foodborne infections in poultry (2). In humans, infections related to *Salmonella* enterica Arizonae associated with birds and chickens have not been specifically reported. Although Arizonosis, associated with reptiles, has been frequently reported in humans (3-6).

The goal of the modern poultry industry worldwide is to achieve high production and better quality at a lower cost. In addition to meeting the increasing demand for poultry meat, the industry requires consistent, efficient, and targeted health care measures to prevent the development and spread of diseases (2). In recent years, the poultry industry in Iran has made significant progress. According to statistics from the Ministry of Jihad Agriculture, in the year 2021, there were 18 turkey breeder units with a capacity of 119,400 birds and 521 commercial turkey units with a capacity of 3,202,958 birds in the country, producing 45,000 tons of meat annually. This growth is remarkable compared to the year 2016, when there were only 9 turkey breeder units with a capacity of 37,610 birds and 324 commercial turkey units with a capacity of 1,703,882 birds. However, given the rapid growth rate and low feed conversion ratio, attention must be paid to gastrointestinal health, especially the intestines, to increase profitability (7).

Arizonosis or avian arizonosis is an acute or chronic disease transmitted from eggs to young turkey poults. Its characteristics include septicemia, neurological symptoms, blindness, and increased mortality. It is caused by the subspecies *Salmonella* enterica Arizonae (*S.* Arizonae). Other bird species such as chicks, ducks, wild birds, canaries, and parrots are also susceptible to infection. In the past, *S.* Arizonae was one of the most common serotypes detected in chickens in the United States (8), leading to significant morbidity and mortality. Serious outbreaks and sporadic cases still occur in this country (9-11). Clinically, avian arizonosis cannot be easily distinguished from other *Salmonella* serotypes such as *S.* Typhimurium and *S.* Heidelberg. Avian arizonosis is economically important in the North American poultry industry and other specific regions worldwide due to increased morbidity and mortality in young turkey poults, reduced egg production in turkey breeder flocks, and decreased hatchability (12-15). The incurred costs include testing birds and eggs, equipment such as swabs, collection of dead birds, reduced egg production, cleaning and disinfection, antibiotic use for treating turkey breeder flocks and poults, and labor costs for disease eradication. Given the limited and insufficient information on *S.* Arizonae contamination in poultry farms and the rapid growth and development of the commercial turkey industry in the country in recent years, there is a need for more comprehensive and extensive research to address this disease. The aim of this study is to determine the prevalence of *S.* Arizonae contamination in commercial and backyard turkey in the provinces of Golestan, Mazandaran, Gilan, and Tehran.

2 Materials and Methods

In this cross-sectional study conducted in spring in 2021, 138 fecal samples from both backyard and commercial turkeys were collected separately using disposable plastic containers. The samples were randomly collected from various locations within the poultry house in the provinces of Tehran, Golestan, Gilan, and Mazandaran. There were no restrictions on the sampling based on breed. The samples were promptly transferred to the laboratory alongside ice.

Table 1. Samples taken by province and production type

Provinces	Backyard turkey	Commercial turkeys	Total samples taken from commercial and backyard turkeys
Golestan	25	36	61
Mazandaran	11	41	52
Gilan	20	54	74
Tehran	3	50	53
Total	59	181	240

2.1 Culture method for isolation of Salmonella

The microbiological culture method for isolating *Salmonella*, followed the global standard procedures (16). The samples were initially incubated at 37°C for 24 hours in selenite cysteine enrichment broth. Subsequently, they were streaked onto selective agar media including chromagar, *Salmonella-Shigella* agar, Xylose Lysine Deoxycholate (XLD) agar, and MacConkey agar for another 24 hours at the same temperature. Suspicious colonies were then confirmed by further culturing in differential media such as Triple Sugar Iron (TSI) agar and urea agar for an additional 24 hours at 37°C. All the culture media used were from Merck, Germany.

2.2 Positive control test

For the purpose of *Salmonella* Enteritidis testing, pure culture was streaked onto MacConkey agar and incubated at 37°C. Subsequently, serial dilutions were performed until a negative 15 dilution was achieved. From all dilutions, 100 microliters were inoculated onto selective agar media including MacConkey, *Salmonella-Shigella*, chromagar, and XLD (Xylose Lysine Deoxycholate) for complete colony distribution. The medium that allowed complete colony counting was selected, and the number of bacteria present in the original sample was calculated. Five hundred microliters of the desired dilution solution were mixed with 5 grams of fecal material, and then inoculated into selenite cysteine enrichment broth for 24 hours at 37°C. Subsequently, this broth was streaked onto four media types mentioned earlier for *Salmonella* growth confirmation. After confirming positive bacterial growth on these media, differential testing was performed on TSI and urea agar for further identification.

3 Results

3.1 Prevalence of Salmonella Arizona

In this study, microbiological culturing methods were employed using enrichment, selective, and differential media to determine the contamination level in backyard and commercial turkey flocks from four provinces: Golestan, Mazandaran, Gilan, and Tehran. All samples were examined for *Salmonella* contamination, and none of them tested positive for this bacterium. However, 22 samples were found to be contaminated with *Proteus*.

3.2 Positive control test

After bacterial growth on the medium for colony counting, the medium that had a colony count between 25-250 was selected for counting. The negative 15 dilution was the only medium suitable for colony counting. The number of colonies in this medium was 73. Based on this number, the final count of bacteria in the original LB solution was calculated to be 7.3×10^{16}.

The final result of fecal cultivation, after pre-enrichment, turned positive for all environments with a negative 15 dilution factor. In all environments, *Salmonella* growth was observed. Additionally, all TSI tubes demonstrated H2S gas production for various K/A cultivation media. Furthermore, all tubes tested negative for the urease enzyme in the urea agar medium.

Figure 1. Typical *Salmonella* colonies from right to left, chrome agar and XLD agar respectively.

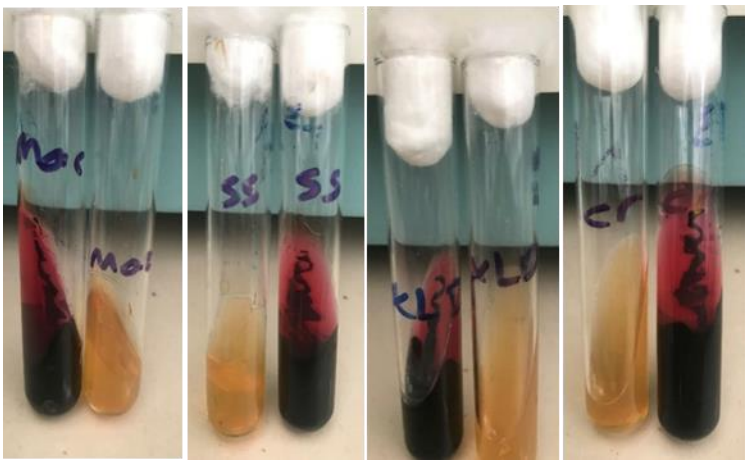

Figure 2. *Salmonella* growth in urea agar and TSI media.

Salmonella was not able to break down urea and the environments remained yellow. With the growth pattern of K/S *salmonella* on TSI medium, the top of the tube will be red and the bottom of the tube will be yellow along with the production of H2S gas.

Figure 3. Typical *Salmonella* colonies from right to left, McConkey and *Salmonella-Shigella*, respectively.

4 Discussion

Salmonellosis is not a reportable disease in Iran. Consequently, the true prevalence of this infection in Iran remains unknown. Data related to the incidence of *Salmonella* should be available for assessment by regulatory authorities as the initial step in controlling salmonellosis in both humans and animals. Therefore, the ultimate goal of this study was to determine the level of *S.* Arizonae contamination in turkey fecal samples.

Salmonellosis is one of the most common causes of foodborne diarrhea worldwide (17), and it remains a significant public health concern in many parts of the world (18). In a study conducted by Walter in the United States in 2000, a food poisoning incident in a family was investigated, and *Salmonella* Enteritidis was successfully isolated from turkey carcasses (19). Fortunately, in this study, the *Salmonella* bacterium was not detected. Some studies conducted in Iran have indicated low contamination rates (0.67% and 2.67%) of *Salmonella* (20, 21), which aligns closely with the findings of the present study. This observation highlights at least minimal concern for public health regarding salmonellosis.

To date, no studies have been conducted on *S.* Arizonae in Iran. The present study is the first official research endeavor to investigate *S.* Arizonae, and due to the fact that no *Salmonella* bacterium has been isolated from fecal samples of both commercial and backyard turkeys, we cannot definitively report the presence or absence of *S.* Arizonae in the country. Further studies are needed in this regard. In a study conducted on 292 samples from reptiles (including turtles, snakes, and lizards) in Croatia, 26 samples (8.9%) were found to be contaminated with various *Salmonella* species. Among these species, *S.* Arizonae was isolated in 6 different samples (2.04%). The highest contamination rate was observed in turtles, with 48.4% (15/31) showing *Salmonella* presence. Additionally, the highest prevalence of contamination (10/41) was found in fecal samples from animals (22). Future studies could explore the transmission characteristics of this bacterium, which is primarily associated with reptiles (such as snakes, turtles, etc.), in other geographical regions. For instance, regions in the central and southern parts of the country, characterized by dry and semi-arid climates, where these reptiles are more abundant.

In comparison to studies conducted on the prevalence and antibiotic sensitivity of *Salmonella* in chickens, clinical and diagnostic efforts related to this bacterium in the context of turkey industry have been significantly limited in Iran. There is scarce data available regarding the incidence of this disease. In interviews with members of the veterinary system, it was revealed that there is no national program for monitoring *Salmonella* in industrial turkeys within the country. This scarcity of attention may be attributed to the relatively nascent state of the turkey industry in Iran. Hopefully, as the industry grows and advances, further research will be conducted to assess *Salmonella* contamination in the country.

Among animal-derived foods, poultry is the primary reservoir for *Salmonella*. Numerous studies have reported the prevalence of this bacterium, and its incidence varies significantly in our country. *Salmonella* contamination ranges from 7.93% in broiler chickens (23) to 19.35% in broiler breeder farms and feed (24), and up to 33.75% in broiler breeder farms (25). Neighboring countries also experience *Salmonella* contamination in poultry farms. A study conducted by Carli and colleagues in Turkey calculated the prevalence of *Salmonella* in broiler and layer farms. Among 697 broiler farm samples, 119 (17.1%) were positive, and among 814 layer farm samples, 151 (18.5%) were contaminated with *Salmonella* (26). Given the high prevalence of *Salmonella* in poultry farms, it is crucial to ensure that these birds do not transmit contamination to turkey farms. Considering the large number of poultry farms compared to turkey farms in our country, and the interconnectedness between these farms, maintaining biosafety measures is essential to prevent *Salmonella* transmission to *Salmonella*-free turkey farms.

In the present study, none of the 240 fecal samples from commercial and backyard turkeys yielded any isolated *Salmonella* bacteria. The results obtained in this study differ from some of the limited studies conducted on turkeys in the country. To assess the prevalence of *Salmonella* in raw turkey meat, ostrich, and quail collected from retail markets, a study was conducted (27). Out of 144 samples of raw turkey meat, 14 samples (9.7%) were contaminated with *Salmonella*. It is possible that cross-contamination occurred during sample collection from these retail markets, either at the time of sampling or before it, resulting in the transfer of *Salmonella* bacteria from poultry meat and other poultry products to turkey meat. Another study examined the prevalence of *Salmonella* contamination in slaughterhouse residues (liver, gizzard, and heart) of turkeys, chickens, and ostriches in retail markets. Out of 105 turkey samples, 7 samples (6.7%), and out of 150 chicken samples, 14 samples

(9.3%) were contaminated with *Salmonella* (28). This study was conducted in Shahrekord and Isfahan, and the discrepancy with the present study may be due to differences in geographical location, cross-contamination in the slaughterhouse, and different sample types. Another study with different findings from the present study was conducted by Jahantigh and colleagues at Zabol University in 2015, in which 8.14% of the samples were contaminated with *Salmonella* (29). Since the methodology of the present study was consistent with this study, the difference in prevalence is likely due to geographical variation. The study does not specify whether the samples belonged to commercial farms or were obtained from native turkeys; however, the possibility of *Salmonella* prevalence in backyard turkeys due to simultaneous rearing with other birds, similar to chickens, may be higher and increase the chances of *Salmonella* isolation.

The birds sent to slaughterhouses can carry both pathogenic and non-pathogenic bacteria on their skin, feathers, and in their intestinal contents. These bacteria can be transmitted to other carcasses during various stages of poultry meat production (30). In the poultry slaughter process, water used in the evisceration, pre-chilling, and chilling sections can facilitate mutual transfer of bacteria (31). Currently, *Salmonella* contamination in poultry is a global concern. Birds that test positive for *Salmonella* can carry high levels of microorganisms in their feces and external coverings during slaughter, leading to secondary contamination at various stages of the process. This contamination can affect the environment, tools, equipment, and ultimately the final product (32). In a study conducted by Niazi to quantitatively and qualitatively assess *Salmonella* contamination in poultry carcasses from industrial slaughterhouses in Tehran province, 61% of the samples showed contamination with *Salmonella* (33). Another study by Zali and colleagues aimed to compare *Salmonella* contamination in different organs (heart, liver, ovary) and feces of slaughtered poultry in the Urmia industrial slaughterhouse. Out of 1440 samples, 300 (20.83%) were contaminated with *Salmonella* (34). Jamshidi and Naghdipour investigated the contamination of water used in the poultry carcass cooling system with *Salmonella* species in the Mashhad industrial slaughterhouse. Among 52 samples, 10 cases (19.23%) were contaminated with *Salmonella*. The study identified 9.1% related to *Salmonella* Typhimurium, 8.5% to *Salmonella* Enteritidis, and 5.11% to other *Salmonella* serovars (35). Another study by Honari and colleagues evaluated non-specific defense measures to reduce *Salmonella* contamination in poultry carcasses from industrial slaughterhouses in Tehran province. The contamination rates in the years 93, 94, and 95 were estimated at 33%, 22%, and 2%, respectively. The highest contamination was attributed to *S*. Enteritidis and *S*. Infantis (36). Given the lack of dedicated slaughterhouses for turkeys, cross-contamination may occur, as mentioned earlier. *Salmonella* from contaminated birds, such as chickens, could be transmitted to turkey carcasses, leading to false contamination in turkeys. Even if the slaughter is not simultaneous, *Salmonella* may persist in the environment and be transferred to turkey carcasses.

Studies conducted in Zimbabwe and Brazil on 2833 samples of slaughtered chickens and 4581 samples from non-human sources reported a prevalence of less than 1% for *S*. Arizonae (37, 38). Another study aimed to assess the prevalence of *Salmonella* in pig farms, dairy and beef cattle, broilers, layers, and turkeys. The contamination rate with *Salmonella* in poultry farms was 16.2%. In this study, *S*. Arizonae was not isolated from poultry farms (39). In a study by Lamas and colleagues (40) to isolate *Salmonella* Arizonae in broiler chicken farms, out of 6577 samples collected from 371 farms, only 19 samples were contaminated with *S*. Arizonae, indicating a low contamination level. It appears that important serovars of *S*. enterica Arizonae have been largely or completely eradicated from European turkey production farms (41, 42). In a baseline study of *Salmonella* in community-level turkey production conducted in 2006-2007, *S*. enterica Arizonae was not observed. Recently, there is no evidence of *S*. Arizonae isolation in EU Trends and Sources reports, as well as reports related to *Salmonella* in British flocks (43). Considering the studies conducted, it seems that the prevalence of this species of *Salmonella* is very low worldwide and may even be eradicated in many regions. However, future studies should involve a large number of samples to increase the chances of isolating *Salmonella* Arizonae.

Salmonella contamination in broiler breeder hens can lead to vertical transmission of the bacterium to broiler chicks through eggs, making it a significant source of contamination in commercially raised meat chicks (44). Studies have also been conducted in this field within the country, reporting contamination in broiler breeder farms (24, 25). These studies may indicate the potential transmission of *Salmonella* from breeder hens to commercial chicks and justify the high prevalence of *Salmonella* in commercial poultry. In a study conducted in Germany on

broiler breeder flocks, out of 6 breeder flocks, 4 (66.7%) were contaminated with *Salmonella* among 24 broiler meat farms. Among these, 7 flocks (29.2%) were contaminated with a single serovar, and 9 flocks (37.5%) were contaminated with two or more serovars of *Salmonella* (45). Another study in France by Aury and colleagues examined *Salmonella* contamination in both breeder and turkey flocks. The prevalence of *Salmonella* was 1.5% in breeder flocks and 15.6% in turkey flocks (46). In the United Kingdom, a comprehensive study was conducted on turkey breeder and commercial turkey farms at all stages of production. Initial sampling revealed contamination rates of 8.9% and 32.8% for breeder and commercial turkey flocks, respectively (47). Although the number of breeder turkey farms in the country is limited, one-day-old chicks are imported, and acceptable control measures are in place for the import of breeder poults, and periodic monitoring for *Salmonella* is performed. To date, no reports of *Salmonella* contamination in breeder broiler farms have been documented in the country. Given that vertical transmission is one of the most important routes for *Salmonella* spread, and breeder turkey farms in the country are considered free from this bacterium, the likelihood of *Salmonella* isolation from well-maintained commercial farms may be very low, similar to the findings of the present study.

Considering the absence of *Salmonella* contamination in fecal samples collected from both commercial and backyard chickens in the northern provinces and Tehran, as determined by microbiological methods in this study, it can be concluded that the hygiene level in commercial poultry farms is acceptable and free from *Salmonella*. However, further studies are needed in this area. These studies could employ different isolation methods (such as PCR) and collect a larger number of samples to investigate poultry farms in other regions with varying ecological and geographical conditions. Hopefully, as the poultry industry in the country advances, more comprehensive research will enhance our knowledge, similar to the progress seen in the poultry industry.

Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

MKH drafted the manuscript, and SMP critically reviewed and revised it. Both authors have read and approved the final manuscript and agreed to the published version of the manuscript.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

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