Original article Casp. J. Vet. Sci., 2026; 3(1): 45-51

Amol University of Special
Modern Technologies

Caspian Journal of Veterinary Sciences

doi: 10.22034/cjvs/2026.565800.1054

Journal homepage: https://Cjvs.ausmt.ac.ir/

Microbial investigation of drinking water in broiler farms for *Salmonella* and *Escherichia coli* bacteria using the most probable number method in northern Khuzestan, Iran

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Article Info

Article history:

Submit Date: 13 December 2025

Accept Date: 25 April 2026

Online Date: 17 June 2026

Keywords:

Drinking water

Escherichia coli

Khuzestan

Poultry farms

Salmonella

Abstract

Microbial contamination of drinking water in poultry production poses significant risks to bird health and public safety. This study aimed to evaluate the microbial quality of drinking water in broiler farms in northern Khuzestan, Iran, focusing on *Escherichia coli* and *Salmonella* prevalence. A cross-sectional study was conducted from July to October 2024, analyzing 32 farm water samples and 10 municipal water sources (controls) using the Most Probable Number (MPN) method. *E. coli* was detected in 59.4% of farm samples (mean: 85 MPN/100 ml), significantly higher than the 10% contamination in municipal water (mean: 0.8 MPN/100 ml). A significant positive correlation was found between water temperatures above 25°C and *E. coli* counts ($r = 0.55$, $p = 0.03$), suggesting that environmental heat accelerates bacterial proliferation. *Salmonella* was detected in 6.25% of farm samples and was absent in municipal water. The results revealed major vulnerabilities in farm water management compared to the effective centralized municipal treatment. While *E. coli* remains a reliable fecal indicator, the presence of *Salmonella* underscores the need for improved pathogen surveillance. Implementing infrastructural upgrades, farmer training, and routine microbial monitoring are essential to mitigate health risks and enhance biosecurity in the region's poultry industry.

Introduction

Ensuring access to high-quality drinking water is essential in the broiler industry to support optimal growth and prevent the introduction of pathogenic microorganisms. Contaminated water with pathogenic bacteria poses a direct threat to animal health and may result in the production of contaminated poultry products, thereby facilitating the transmission of zoonotic diseases. Among the bacterial pathogens of greatest concern, *Salmonella* and *Escherichia coli* serve as critical indicators of water safety. *Salmonella* is a resilient pathogen capable of surviving for extended periods in various environments, posing a persistent risk to the poultry production chain (Djeffal *et al.,* 2025). Conversely, *E. coli* is a standard indicator of fecal contamination, and its presence often correlates with the occurrence of systemic infections and significant economic losses in the poultry sector (Swelum *et al.,* 2021; Yousef *et al.,* 2023).

The high prevalence of respiratory diseases in poultry causes substantial economic losses to the poultry industry each year. Among these conditions, bacterial infections, particularly those caused by *E. coli*, play a major role in the occurrence and severity of these problems (Yousef *et al.,* 2023).

In this study, the microbial contamination of drinking water in broiler farms located in northern Khuzestan was assessed with a focus on *Salmonella* and *E. coli*. To accomplish this, the Most Probable Number (MPN) method was employed. This method, which is based on estimating the numerical concentration of bacteria in water samples, provides an accurate evaluation of the sanitary status and quality of drinking water. The findings of this research can serve as a basis for implementing improved water management practices and reducing microbial contamination in the broiler production industry within the region.

Materials and Methods

Study design and sampling

This descriptive-analytical cross-sectional study was conducted between July and October 2024 to evaluate the microbial quality of drinking water in northern Khuzestan, Iran (encompassing Shoushtar and surrounding districts). A total of 32 active broiler farms were selected, all of which relied on independent groundwater wells as their primary water source. This water was typically pumped and stored in ground-level or overhead storage tanks before distribution. Additionally, 10 municipal water samples (piped water from the regional distribution network) were collected from the same geographic area to serve as a control group.

Inclusion criteria for the farms included active production status, the use of independent water storage tanks, and the absence of any water disinfectants or antimicrobial treatments within one week prior to sampling to ensure an accurate assessment of the baseline microbial load. Sampling was performed during the peak high-temperature season to evaluate water quality under maximum environmental microbial stress.

A total of 42 water samples (500 ml each) were collected between 8:00 and 10:00 a.m. in sterile bottles containing 10% sodium thiosulfate (0.1 ml) to neutralize residual chlorine. Physicochemical parameters, including temperature and pH, were measured on-site. All samples were transported to the laboratory at 4°C and processed within 6 hours of collection.

Microbiological analysis

The Most Probable Number (MPN) technique was employed for the quantification of *E. coli* and *Salmonella spp.* according to standard protocols (APHA, 2017).

For *E. coli* detection, samples were first inoculated into Lauryl Sulfate Tryptose (LST) broth (Merck, Germany) for presumptive enrichment. Tubes showing gas production and turbidity were transferred to *E. coli* (EC) broth (Merck, Germany) and incubated at 44.5°C for 24 hours. Positive EC tubes were subsequently streaked onto Eosin Methylene Blue (EMB) agar. Characteristic colonies (black centers with metallic green sheen) were confirmed as *E. coli* using IMViC biochemical tests (Indole, Methyl Red, Voges-Proskauer, and Simmons Citrate).

For *Salmonella* isolation, a three-stage procedure was followed:

1. Pre-enrichment: 25 ml of each water sample was added to 225 ml of Lactose Broth (Merck, Germany) and incubated at 37°C for 48 hours.

2. Selective enrichment: 1 ml of the pre-enriched culture was transferred into Tetrathionate Broth and Selenite Cystine Broth (Merck, Germany) and incubated at 37°C for 24 hours.

3. Selective plating and confirmation: Enriched cultures were streaked onto *Salmonella*-specific selective media (XLD or SS agar). Suspected colonies (colorless with black centers) were validated using Triple Sugar Iron (TSI) agar, SIM medium, Urea agar, and MR-VP tests.

Statistical analysis

Quantitative data were analyzed using SPSS (v. 24.0) and GraphPad Prism. Normality of data was assessed using the Shapiro-Wilk test. Differences between farm and municipal groups were analyzed using t-tests or Mann–Whitney U tests. Results were expressed as mean ± standard deviation (SD), and a *p*-value < 0.05 was considered statistically significant.

Results

The results of this study indicated that drinking water used in broiler farms in northern Khuzestan was significantly contaminated with *E. coli*, whereas *Salmonella* contamination was very limited.

Among the 32 water samples collected from farm reservoirs, the mean *E. coli* concentration was 85 MPN/100 ml (± 30 SD). Approximately 59.4% of farm samples (19 out of 32) tested positive for this bacterium (Table 1, Fig. 1).

Table 1. Results related to *Escherichia coli* bacteria in poultry tank samples.

Sample group	Average bacteria per 100 ml	Standard deviation	Positive samples (%)
Poultry farm tanks	85	30±	19 (59.4%)

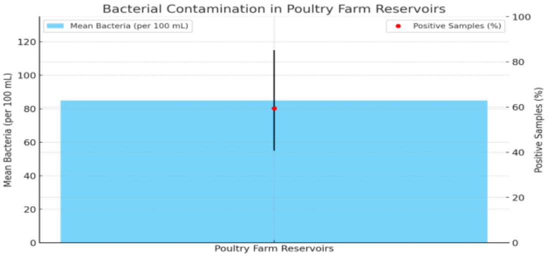

Fig. 1. Results related to *Escherichia coli* bacteria in poultry tank samples.

In contrast, municipal drinking water samples exhibited a very low mean *E. coli* contamination of 0.8 MPN/100 ml (± 0.5 SD), with only one sample (10%) testing positive (Table 2, Fig. 2).

Table 2. Results related to *Escherichia coli* bacteria in municipal water.

Sample group	Average bacteria per 100 ml	Standard deviation	Positive samples (%)
Municipal drinking water	0.8	0.5±	1 (10%)

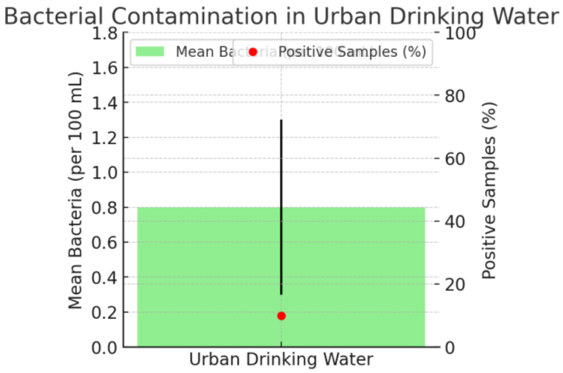

Fig. 2. Results related to *Escherichia coli* bacteria in municipal water.

A t-test confirmed a statistically significant difference between the two groups ($p < 0.001$), indicating that contamination in broiler farm water was 106 times higher than in municipal sources. The highest *E. coli* concentrations were observed in larger farms. Additionally, a significant positive correlation was found between water temperature (> 25°C) and increased *E. coli* levels ($r = 0.55$, $p = 0.03$).

Regarding *Salmonella*, the situation was markedly different. Only 2 out of 32 farm samples (6.25%) tested positive, with a mean concentration of 1.9 MPN/100 ml (± 7.6 SD) (Table 3, Fig. 3).

In contrast, none of the municipal water samples tested positive for *Salmonella*. The difference between farm and municipal water was statistically significant ($p < 0.05$). Further analyses demonstrated no statistically significant correlation between the presence of *Salmonella* and physicochemical parameters, including pH ($r = 0.14$, $p = 0.46$) and Total Dissolved Solids (TDS) ($r = 0.09$, $p = 0.62$).

Discussion

This study provides critical insights into the microbial quality of drinking water in broiler farms in northern Khuzestan, Iran, highlighting significant differences between agricultural and municipal water systems. The high prevalence of *E. coli* in farm water, compared to its minimal presence in municipal sources, underscores systemic weaknesses in farm water management. *E. coli*, a well-established indicator of fecal contamination, was detected in approximately 60% of farm samples, reflecting ongoing gaps in hygiene practices, infrastructure maintenance, and water treatment protocols. The positive correlation between elevated water temperatures and increased microbial load aligns with microbial growth kinetics, as warmer environments accelerate bacterial metabolism and biofilm formation. This finding is particularly relevant in Khuzestan's hot and dry climate, where water stored in open or poorly insulated tanks provides an optimal environment for pathogen proliferation.

In contrast, *Salmonella* was detected in only 6.25% of farm water samples, presenting a notable disparity given its frequent association with poultry in global studies. This limited occurrence may be explained by ecological competition, where *E. coli* and other microbiota outcompete *Salmonella* in nutrient-rich environments, or by the pathogen's higher sensitivity to environmental stressors, such as residual chlorine, UV exposure, or pH fluctuations. Both *Salmonella*-positive farms lacked effective filtration systems, suggesting that contamination likely originated from external vectors (e.g., rodents, contaminated feed, or human activity) rather than in situ proliferation. This observation is consistent with prior studies indicating that *Salmonella* survival in water systems often depends on specific hosts or direct fecal-oral pathways, whereas *E. coli* can persist and grow independently due to its biofilm-forming capacity and environmental resilience.

The stark contrast between farm and municipal water quality highlights the effectiveness of municipal water treatment systems, which typically employ chlorination, filtration, and routine monitoring to reduce contamination. In contrast, farms in this study relied on untreated or poorly maintained water sources, such as groundwater stored in aging tanks. The lack of enforceable standards for agricultural water quality exacerbates these risks, particularly in areas with intensive poultry farming. Large-scale farms, which exhibited higher microbial loads, face additional challenges: high bird density strains water distribution systems, promoting stagnation and biofilm formation, while logistical constraints in scaling hygiene measures, such as routine tank cleaning or disinfection, further increase contamination risks. These observations reflect global trends in industrial poultry production, where infrastructure and management deficiencies often compromise water safety.

The underlying biological mechanisms further elucidate these risks. The dominance of *E. coli* in farm water can be attributed to its ability to exploit organic matter from poultry waste, feed residues, and biofilms, creating persistent contamination reservoirs. Its biofilm-forming capacity protects it from residual disinfectants and temperature fluctuations, enabling long-term survival in storage systems. In contrast, *Salmonella* lacks similar environmental resilience, often relying on protective hosts or organic particles, making it more susceptible to die-off in open water systems. This ecological dynamic explains why *E. coli* serves as a reliable indicator of hygiene failures, whereas *Salmonella* presence signals acute breaches in sanitation, such as cross-contamination from external sources.

Methodologically, the use of MPN approach provided a practical framework for estimating bacterial concentrations, though its probabilistic nature introduces inherent uncertainty, especially at low contamination levels. Inclusion of urban control samples and adjustment for physicochemical factors (e.g., pH, TDS) enhanced the validity of comparisons, while biochemical confirmation reduced false positives. However, focusing on central water tanks rather than end-use points (e.g., drinkers) may underestimate localized contamination from biofilm-coated pipes or feeder outlets. Additionally, the cross-sectional design limits the ability to assess temporal trends, such as seasonal microbial fluctuations or the impact of flock turnover on water quality.

These findings carry significant implications for public health and agricultural policy. High levels of *E. coli* in farm water pose direct risks to poultry health, potentially increasing susceptibility to infections, reducing growth rates, and raising mortality, all of which have economic consequences for farmers. Indirectly, contaminated water can contribute to the development of antimicrobial resistance (AMR) by fostering the proliferation of resistant strains that may transfer to humans through the food chain or environmental exposure. Addressing these challenges requires a multifaceted approach: routine water testing and biofilm removal, upgrading outdated infrastructure with filtration or UV systems, and establishing enforceable standards for agricultural water quality. Educational programs for farm workers on hygiene practices and water safety can further mitigate these risks.

Overall, this study provides important insights into the prevalence of *E. coli* and *Salmonella* in broiler farm water in northern Khuzestan, Iran. The findings can be contextualized within previous research in Iran and internationally, highlighting both consistencies and discrepancies arising from methodological, ecological, and managerial factors. The observed high prevalence of *E. coli* (approximately 59.4%) aligns with other Iranian studies-for example, Ashrieh *et al.* (2009) reported 78% contamination in poultry farm water in Qaemshahr, and Soudeh *et al.* (2012) identified *E. coli* in 60% of colibacillosis cases in Ahvaz. These parallels point to a systematic issue within the Iranian poultry industry, driven by inadequate water hygiene, aging infrastructure, and environmental stressors such as elevated temperatures. Moreover, the positive association between water temperatures above 25°C and *E. coli* growth observed in this study corroborates findings by Soudeh *et al.,* (2012) on enhanced bacterial proliferation under warmer conditions.

However, compared to studies conducted outside Iran, notable differences are evident. For instance, Tagenge *et al.,* (2024) reported only 5.2% contamination with *E. coli* O157:H7 in abattoir samples in Kenya, whereas Bratfeln *et al.,* (2023) found a 30% prevalence of *E. coli* in poultry farms in Romania. The lower prevalence in some studies may be attributed to methodological differences; for example, Tagenge *et al.* (2024) focused exclusively on the pathogenic serotype O157:H7, while the present study assessed total *E. coli* as a general indicator of fecal contamination. Additionally, stricter biosecurity protocols in abattoirs compared with farms, as well as regional variations in water sources (e.g., groundwater versus treated municipal water), may contribute to these observed differences (Brătfelan *et al.,* 2023) and (Tegegne *et al.,* 2024).

The low prevalence of *Salmonella* (6.25%) observed in the present study contrasts with higher rates reported in global studies. For example, Jamshidi and Naghdi-Pour (2011) detected *Salmonella* in 19.23% of water samples from abattoir cooling systems in Mashhad, primarily comprising *S. Typhimurium* and *S. Enteritidis*.

The low prevalence of *Salmonella* in this study may be attributed to ecological competition; *E. coli*, being a more resilient and fast-growing bacterium, may outcompete *Salmonella* in nutrient-rich water systems. This hypothesis is supported by Youssef *et al.* (2023), who highlighted the high survival and proliferation capacity of *E. coli* across diverse environmental conditions.

In addition, methodological factors likely influenced the findings. In the present study, the MPN method was employed to estimate bacterial concentrations. While this approach is standard for water analysis, it may underestimate *Salmonella* prevalence due to the viable but non-culturable (VBNC) state or intermittent shedding by poultry. In contrast, Jamshidi and Naghdi-Pour (2011) utilized PCR to detect specific *Salmonella* serotypes, providing higher sensitivity and accuracy. Furthermore, the two farms that tested positive for *Salmonella* lacked functional filtration systems, suggesting that contamination likely originated from external sources, such as rodents or poultry feed, rather than in situ bacterial proliferation in water.

The pronounced difference between farm water (85 MPN/100 ml *E. coli*) and municipal water (0.8 MPN/100 ml) observed in this study corroborates the findings of other studies. This contrast highlights the effectiveness of municipal water treatment systems, such as chlorination and filtration, compared to unregulated or poorly treated farm water sources, including underground storage tanks.

High contamination levels in larger farms (over 20,000 birds) align with the results of Karimi *et al.* (2011), who reported a direct correlation between *E. coli* levels in water and colibacillosis prevalence in Qom poultry farms. Larger farms face greater challenges in maintaining water hygiene, including biofilm accumulation in aging pipes and irregular disinfection schedules, a problem exacerbated in hot, dry regions like Khuzestan.

Although this study did not evaluate antimicrobial resistance (AMR), the findings relate to those of Karimi *et al*. (2011), who reported high tetracycline resistance in *E. coli* isolates from poultry in Qom. Similarly, Bratfeln *et al*. (2023) identified multidrug-resistant *E. coli* in Romanian poultry farms, reflecting a global trend of increasing antibiotic resistance in livestock. The dominance of *E. coli* in farm water, combined with its potential to carry virulence genes (e.g., *stx*, *eae*), represents both a zoonotic and antimicrobial threat-an issue further aggravated by the absence of stringent water quality standards for agricultural settings (Brătfelan *et al.,* 2023).

Although the MPN method used in the present study is a standard approach, it carries inherent probabilistic uncertainty, particularly at low contamination levels. Similar challenges were reported by Ashrieh *et al.* (2009) and Karimi *et al.,* (2012), whereas Yusuf *et al.* (2023) and Jamshidi and Naghdi-Pour (2011) employed molecular techniques, such as PCR, to achieve greater detection accuracy (Ashrieh *et al.,* 2009) and (Yousef *et al.,* 2023).

This study provides valuable insights into the microbial quality of drinking water in broiler farms in northern Khuzestan, Iran, highlighting significant differences between agricultural water systems and municipal sources. The findings reveal widespread contamination of farm water with *E. coli*, with a prevalence of 59.4% among samples, in stark contrast to the negligible levels observed in municipal water. This disparity reflects systemic deficiencies in farm water management, including inadequate hygiene practices, aging infrastructure, and the absence of standardized treatment protocols. Moreover, the observed correlation between elevated water temperatures and increased *E. coli* concentrations underscores the vulnerability of stored water in hot and arid regions such as, Khuzestan, where environmental conditions promote microbial growth.

In contrast, the low prevalence of *Salmonella* (6.25%) suggests that ecological or methodological factors may limit its detection. This low incidence could result from competitive exclusion by dominant bacteria such as *E. coli* or the higher environmental sensitivity of *Salmonella*. Nonetheless, it underscores the need for molecular approaches to complement culture-based techniques like MPN. The two farms with positive *Salmonella* samples lacked effective filtration systems, highlighting the role of external contamination sources, such as rodents or feed, in the sporadic introduction of pathogens.

Acknowledgment

The authors are grateful to the microbiology staff of Shoushtar Azad University for supporting the current study.

Conflict of Interests

The authors declared no conflict of interest.

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