University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 206-212

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/6wmrx052

Molecular detection of Salmonella bacteria in laying hens using Real-Time PCR and its impact on productive traits

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Abstract

This study aimed to evaluate the prevalence of Salmonella spp. Within the laying hen flocks, using a Real-time PCR reaction that affects the invA gene, in addition to studying the morphological and internal characteristics of deformed eggs compared to normal eggs, 100 blood samples were collected from the laying hens, and the PCR results showed that 55% of the flock were infected with Salmonella bacteria, indicating the spread of the disease. Also, 100 egg samples were analyzed, including 75 deformed eggs and 25 normal eggs. Its external and internal characteristics were analyzed, and the results were presented using descriptive statistics and t-test analysis. The analysis showed that the differences between deformed and normal eggs were statistically significant with respect to egg length, egg width, shell thickness, and yolk weight ($P \geq 0.001$), while the differences in egg weight and albumen weight were not statistically significant ($P > 0.05$). These results highlight the importance of using molecular polymerase chain reaction for the rapid detection of infected cases and highlight the potential impact of infection on egg quality. The research concluded that there is a widespread prevalence of the disease in laying hen farms, emphasizing the need to conduct periodic infection and egg quality checks in order to improve production health and food safety.

Keywords \ Salmonella spp-InvA gene-Real-Time PCR-Layer chickens-Egg quality

I. Introduction:

Salmonella is considered one of the most dangerous types of bacteria that infect chicken flocks and affects food safety and public health, as the infection spreads through the food supply chain from the farm to the consumer and causes a decrease in productivity in laying hens and contamination of eggs. Previous scientific studies have also shown its widespread spread in poultry fields, especially within strains such as Enteritidis and Typhimurium, with the infection rate remaining relatively high. This finding underscores the importance of implementing targeted molecular detection methods, particularly PCR assays directed at specific virulence-associated genes such as invA, as rapid and accurate tools for identifying the pathogen in clinical and environmental samples. Such approaches play a critical role in strengthening prevention and control strategies aimed at limiting the spread of infection throughout poultry production systems (Barrow and Methner, 2013) (Eng et al., 2015) (Waktole et al., 2024).

In addition to microbial diagnosis, the bird's immune response remains a central factor in determining the degree of chicken resistance to pathogenic bacterial infection, especially in cases of chronic infections that can affect immune system functions and lead to reduced productivity. Recent studies have shown that the primary task of the immune system in poultry is to coordinate the responses of both innate and acquired immunity, which requires activation of complex interactions of lymphocyte cells, including T cells and B cells. Recent evidence suggests that the pathogen-responsive molecular network includes dynamic expression of a range of immune genes that control the balance between effective response and control of inflammation, making understanding of these interactions essential to improving birds' resistance to bacterial diseases. (Kogut, 2019) (Abbas et al., 2021) (Santiago et al., 2025)

Previous research has indicated that the invA gene is effectively used for molecular detection of Salmonella bacteria in biological samples. This gene is consistently present in pathogenic strains of Salmonella, making

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it a suitable target in polymerase chain reaction (PCR) assays for diagnostic purposes. One study also showed that the detection rate of the invA gene was high in isolates taken from poultry environments, which confirms its important role in molecular surveillance. (Salem et al., 2025) (Salehi et al., 2005; Mohammed, 2022).

On the other hand, cytokines play a pivotal role in regulating the immune response, as these signaling molecules are essential elements in activating and directing immune cells during the confrontation with pathogens. Although there are few direct studies on the IL-2 gene in poultry compared to ruminants, available evidence from recent immunological research indicates that IL-2 contributes to regulating T-cell proliferation and its interaction with other immune pathways, which affects the efficiency of cellular defense against pathogens such as Salmonella. Recent studies have also shown that stimulation of T cells by IL-2 can enhance the expansion of γδ T cells, which represent an important component of innate immunity in chickens. This highlights how cytokines can support the immune response in birds (Antonia et al., 2024)(Hussein et al., 2020; Brisbin et al., 2008).

Modern layer production environments face multiple challenges including environmental factors, feeding system design, and farm management, all of which may affect the birds' overall immunity and specifically their ability to resist bacterial infections. Recent research suggests that improving environmental conditions, combined with genetic and nutritional strategies, can enhance the immune response and support production efficiency, highlighting the need for a comprehensive approach that integrates molecular diagnostics, environmental variables and genetic immunity into the strains used for egg production. (Santiago et al., 2025)(Hofmann & Schmucker, 2020; Pinto & de Souza, 2024).

Estimates indicate that approximately 1.35 million people are infected with Salmonella each year, resulting in around 420 deaths according to the Centers for Disease Control and Prevention, The economic burden associated with Salmonella ranks third among illnesses caused by foodborne pathogens annually, with an estimated cost of about $3.3 billion per year (Balasubramanian et al., 2019)(Scallan et al., 2011; Hoffmann et al., 2025).

Furthermore, some studies have shown that infection of laying hens with S. enteritidis led to a 33.33% reduction in egg production compared with healthy birds. It was also found that 60% of the birds were infected with the bacterium within 61 days after infection, indicating a clear negative impact on the productive performance of laying hens (Khatun et al., 2022)(Zhang et al., 2019; Andreatti Filho et al., 2019).

II. Materials and Methods

Sample Collection : 75 blood samples were collected from the jugular vein of infected chickens, with 5 ml drawn from each bird. Samples were gathered from various farms in Thi-Qar Governorate in 2025. Additionally, 25 blood samples were collected from healthy chickens as a control group. Samples were transferred to EDTA (Ethylene diaminetetraacetic acid) tubes to prevent coagulation. The samples were immediately transported to the laboratory in cooled containers to ensure sample preservation.

Safety Precautions :All procedures were performed following biosafety guidelines, including wearing gloves, masks, and using disinfectants to minimize infection transmission risk.

Detection of Salmonella :Salmonella detection was performed using Real-time PCR targeting the invA gene.

Measurements of Deformed Eggs

Samples of deformed eggs were collected, and their weight was measured using a sensitive electronic balance. Egg length and width were determined using a vernier caliper. In addition, yolk and albumen weights were recorded after breaking the egg, and shell thickness was measured using a vernier caliper.

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DNA Extraction from Blood Samples

DNA was extracted from blood samples using the gSYNC™ DNA Extraction Kit, following the manufacturer's instructions.

Amplification of invA Gene using Real-Time PCR

Real-Time PCR was performed to detect the invA gene of Salmonella spp. using the Stratagene Mx3000P Real-Time PCR System.

Reaction Mixture: Extracted DNA - Forward and reverse primers - Master mix - Deionized water

Thermal Cycling Conditions:

- Initial denaturation: 94°C for 10 minutes - 40 cycles:

  - Denaturation: 94°C for 15 seconds

  - Annealing: 60°C for 1 minute

  - Extension: 72°C for 25 seconds

- Melting curve: 65-90°C, 1 cycle

Primers Used for the invA Gene

Specific primers were employed for the detection of the invA gene, as illustrated in Table (1).

Gene	Primer sequence (5′–3′)	Product Size (bp)
InvA	F:5′ACAGTGCTCGTTTACGACCTGAAT-3′	243
	R:5′-AGACGGCTGGTACTGATT ATAAT-3′	

Statistical Analysis : The data were analyzed using SPSS/Excel software and the results were presented as mean ± standard deviation.

Results and Discussion

A comparison was conducted between the external and internal characteristics of 100 samples of normal and deformed eggs. The results showed clear differences in most traits; the average weight of deformed eggs decreased to (55.74 g) compared to (59.27 g) in normal eggs, and a decrease in average length to (5.3 cm) was also observed. The most significant impact was seen in shell thickness, which dropped to (0.24 mm) in deformed eggs, confirming that deformation significantly affects both the morphological and internal characteristics of the egg, as illustrated in Table (2).

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Compare the internal and external characteristics of both normal and deformed eggs Table (2) .

Trait	Normal eggs (Mean ± SD)	Deformed eggs (Mean ± SD(
Egg weight (g)	2.544484 ±59.276	18.2328 ±55.74667
Egg length (cm)	2.544484 ± 5.668	0.804884 ± 5.3
Egg width (cm)	0.150886 ± 4.388	0.512809 ± 4.06
Shell thickness (mm)	0.017795 ±0.36	0.117262 ± 0.245946
Yolk weight (g)	1.064393 ± 18.572	4.703965 ± 16.53425
Albumen weight (g)	1.435061 ±34.776	9.940768 ± 34.71233

Statistical analysis using the T-test revealed that the differences in shell thickness, egg length, egg width, and yolk weight were statistically significant ($P < 0.001$) between normal and deformed eggs. In particular, the significant reduction in shell thickness indicates that deformities directly impair the shell's structural integrity, which is a critical factor in protecting egg contents from microbial contamination. Conversely, no significant differences were observed in total egg weight and albumen weight ($P > 0.05$), as detailed in Table (3).

Statistical Analysis and T-test for Egg Traits Table (3).

Trait	Normal eggs (Mean ± SD)	Deformed eggs (Mean ± SD)	T-value	P-value	Interpretation
Egg weight (g)	2.544484 ± 59.276	18.2328 ± 55.74667	-1.62944	0.107	Not significant
Egg length (cm)	2.544484 ± 5.668	0.804884 ± 5.3	-3.59786	0.001	significant
Egg width (cm)	0.150886 ± 4.388	0.512809 ± 4.06	-4.93528	0.001	Highly significant
Shell thickness (mm)	0.017795 ± 0.36	0.117262 ± 0.245946	-8.09559	0.001	Highly significant
Yolk weight (g)	1.064393 ± 18.572	4.703965 ± 16.53425	-3.45218	0.001	significant
Albumen weight (g)	1.435061 ± 34.776	9.940768 ± 34.71233	-0.05313	0.958	Not significant

This result is consistent with the study by (Messens et al., 2005), which showed that a decrease in shell thickness increases the likelihood of the egg being affected by microbes, especially under stress or infection conditions. As for other characteristics, such as egg weight, length, yolk weight, and albumin weight, no statistically significant differences were recorded. However, descriptive trends indicate that deformed eggs tend to be lighter and smaller compared to healthy eggs. This can be attributed to several factors, including nutrition, the bird's health and immune status, or the presence of a latent bacterial infection, all of which are factors mentioned in the scientific literature as affecting egg quality.

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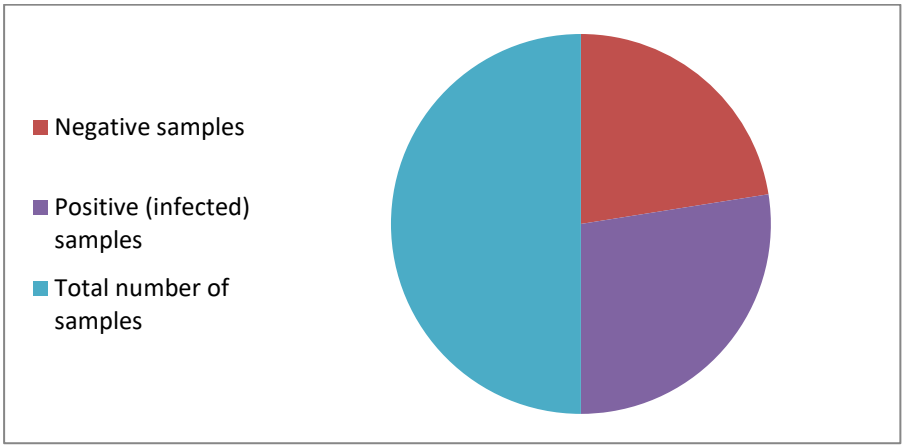
Additionally, the results of the Real-Time PCR indicated that 55% of the examined blood samples were positive for the invA gene of Salmonella spp., while 45% were negative, as shown in Figure 1, reflecting a notable prevalence of the bacteria in the studied layer chicken flocks.

Figure 1: Real-Time PCR results for the invA gene in blood samples.



Compared to our study, which showed that 55% of the samples were positive for the invA gene, a systematic review of multiple studies in Iran showed a prevalence of Salmonella in eggs, with an overall contamination rate of approximately 13.61% in eggshells alone, with prevalence rates reaching up to 29.06% in some areas (Hosseininezhad et al., 2020). Another study conducted in Ethiopia found the prevalence of Salmonella in chicken eggs to be 19.1%, which is much higher than some previous studies (Mekonnen et al., 2022). A study on chicken eggs showed in Indian that about 4% of the samples were positive for Salmonella using the same target gene and Real-Time PCR, confirming the importance of this technique in the biological monitoring of infections in food animal production (LAKHAN et al., 2013).

These results show that the infection rate may vary significantly depending on the testing method, environmental conditions, and farm management methods, which highlights the necessity of relying on precise molecular techniques in epidemiological follow-up. Beyond diagnostic detection, recent genomic analysis in the same region (Thi-Qar, Iraq) by Hanan et al. (2025) revealed significant micro-variations within the invA gene. Despite a 99% homology with global strains, 19 genetic variants were identified, including deletions at sites A35, T42, and A69, alongside several insertion mutations. These findings suggest that the 55% prevalence rate observed in our study may involve evolving bacterial lineages with specific genetic adaptations, highlighting the necessity of combining routine PCR with continuous genomic surveillance.The Ct values for the positive samples ranged between 13.25 and 33.74, which indicates a clear variation in the amount of bacteria present in the birds. Low values reflect a high bacterial concentration, while high values indicate a relatively lower concentration.

This variation may be related to several factors, including the immune status of birds, level of exposure to the pathogen, and environmental and management conditions in different fields. These results are consistent with what Lakhan et al. (2013) found, where they confirmed that targeting the invA gene using Real-Time PCR is a sensitive and rapid method for detecting Salmonella bacteria in poultry and egg samples, and that prevalence rates may vary depending on the sample source and environmental conditions. The high rate of positive samples in this study compared to some other studies may reflect differences in health conditions or control programs, emphasizing the importance of adopting molecular diagnostic techniques in epidemiological monitoring programs in laying farms.

This study highlights the impact of egg deformities on total production in a large-scale layer farm containing 10,000 hens. Considering the proportion of deformed eggs (55%), approximately 5,500 eggs are expected to be deformed compared to 4,500 normal eggs. The difference in weight between healthy and deformed eggs (58 grams versus 55.3 grams) results in an individual loss of approximately 2.7 grams per deformed egg, which equates to a total loss of approximately 14.85 kilograms across the entire flock. This highlights the

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importance of monitoring egg quality and conducting molecular surveillance for pathogenic bacteria. In general, the results indicate a relationship between changes in some egg characteristics, especially shell thickness, and the possibility of the flock being exposed to biological stress or bacterial infection. The results also confirm that the instant PCR technology targeting the invA gene is a reliable means of rapid and accurate detection of Salmonella bacteria in poultry, which supports the recommendation to implement periodic screening programs that combine molecular evaluation and egg quality control to ensure food safety and reduce health and economic risks.

III. Conclusion

1. The analysis of the morphological and internal characteristics of the eggs revealed descriptive differences between deformed and normal eggs, with statistically significant differences observed in egg length, egg width, shell thickness, and yolk weight ($P \geq 0.001$). This suggests that the deformities may be linked to reduced internal and external quality and increased susceptibility to environmental or microbial influences.

2. The other traits, including egg weight and albumen weight, did not show statistically significant differences ($P > 0.05$). However, descriptive trends indicate that deformed eggs tend to be smaller in size and lighter in weight compared to normal eggs, which aligns with scientific explanations related to nutrition, bird health, and immune status.

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