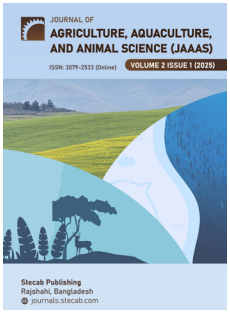

Journal of Agriculture, Aquaculture, and Animal Science (JAAAS)

ISSN: 3079-2533 (Online)

Volume 2 Issue 1, (2025)

https://doi.org/10.69739/jaaas.v2i1.457

https://journals.stecab.com/jaaas

Published by
Stecab Publishing

Research Article

A Study on the Pathological and Bacterial Shedding of 9R *Salmonella gallinarum* and *Salmonella pullorum* Killed Vaccine in Layer Hens

*[1]Hani H. Al-Daraji

About Article

Article History

Submission: March 05, 2025
Acceptance : April 08, 2025
Publication : April 15, 2025

Keywords

ISSA Brown, Most Probable Number, Morbidity, Mortality

About Author

[1] Department of Veterinary Pathology and Poultry Diseases, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

Contact @ Hani H. Al-Daraji
hani.helal2207@covmuobaghdad.edu.iq

ABSTRACT

This study aimed to evaluate the effectiveness of immunization of the 9R *Salmonella gallinarum* strain and killed *Salmonella Pullorum* vaccine in protecting layer chickens from *Salmonella pullorum* infection. 60 chickens were divided into three groups: one group received the 9R vaccine; the other received the killed *Salmonella pullorum* vaccine, and the third group the control negative. after challenge revealed that the group vaccinated with the killed vaccine (G2) noticed slight histopathological changes, represented by moderate MNCs infiltration with heterophils whereas the group that received the live vaccine (G1) showed moderate multiple MNCs infiltration and mild sinusoidal congestion with the prominence of Kupffer cells compared to the third group, which showed focal MNCs infiltration forming granulomatous lesion with sinusoids dilation and congestion. In conclusion, the killed vaccine has an important role in protection against challenges with *S. pullorum* in comparison with the live vaccine (9R), without bacterial shedding, low morbidity without mortality and less histological changes.

Citation Style:

Al-Daraji, H. H. (2025). A Study on the Pathological and Bacterial Shedding of 9R *Salmonella gallinarum* and *Salmonella pullorum* Killed Vaccine in Layer Hens. *Journal of Agriculture, Aquaculture, and Animal Science, 2*(1), 92-98. https://doi.org/10.69739/jaaas.v2i1.457

1. INTRODUCTION

Salmonellosis is a significant bacterial disease affecting poultry worldwide, caused by various Salmonella species (Kabeta *et al*., 2024; Islam *et al*., 2025). Among them, *Salmonella pullorum* and *Salmonella gallinarum* are highly host-adapted to chickens and other gallinaceous birds, leading to two major poultry diseases: pullorum disease and fowl typhoid (Guo *et al*., 2019). Both diseases cause severe economic losses due to high mortality, reduced productivity, and the risk of vertical transmission (Saleem *et al*., 2016). *Salmonella Pullorum* is caused by the non-motile bacterium Salmonella enterica subspecies enterica serovar gallinarum biovar pullorum (Gast *et al*., 2020).

2. LITERATURE REVIEW

PD disease is a severe systemic disease and is associated with a high mortality rate. Infected chickens show a range of symptoms, including anorexia, depression, diarrhea, and persistent cloacal infection (Tian *et al*., 2018). *Salmonella pullorum* can remain in the spleen and reproductive system for several months, even though infected adult hens may not show any symptoms, leading to vertical transmission to eggs and offspring (Shen *et al*., 2022). *Salmonella pullorum* can infect chickens of all breeds and ages. However, mortality rates from infection decrease numerous infected hens get latent and chronic illnesses with age (Revolledo, 2018). Like an intracellular parasite, *S. pullorum* is difficult to eradicate completely, even while medication therapy is enough to control clinical symptoms, ultimately leading to the development of a subclinical persistent infection. After infection, it is clear that *S. pullorum* can modulate host immunity, with antibody responses lasting for more than 40 weeks (Kang *et al*., 2022). The main threat to poultry production in developing countries is PD because it causes significant economic losses every year (Abdhall *et al*., 2023; Eriksson *et al*., 2018). S. gallinarum is closely related to *Salmonella pullorum*, sharing many antigenic and pathogenic features, although each causes distinct diseases (Valentina *et al*., 2022). Fowl typhoid affects poultry of all ages, with adult poultry being particularly susceptible (Vaid *et al*., 2021). Several strategies have been attempted to control the disease in chickens, including vaccination, use of antibiotics, and preventive measures. Vaccination has been accepted to prevent or reduce Salmonella infection in poultry worldwide. Killed vaccines can be effective in reducing Salmonella infection in poultry. They are safe because they do not cause recurrence of the disease, do not spread into the environment, and are considered good enough to protect chickens when used in large-scale poultry production (Buck *et al*., 2004). However, live vaccines are thought to have advantages over killed vaccines with respect to induced immunity (Van Immerseel *et al*., 2005). Live vaccines, by causing the expression of all relevant antigens in vivo, induce better protection against Salmonella because they induce both cellular and humoral immunity and the expression of all relevant antigens in vivo, whereas inactivated vaccines primarily induce the production of antibodies only against antigens present at the time of harvest in vitro (Barrow & Wallis, 2000). Killed vaccines can also be rapidly destroyed and eliminated from the host, and are generally considered incapable of inducing cytotoxic T cell activation (Freitas Neto *et al*., 2007). It is widely accepted that cellular immunity is more important than humoral responses in protection against Salmonella, especially in infections caused by host-specific serotypes (Barrow, 2007). The objective of the study is to assess the most effectiveness immunization of the 9R strain of *Salmonella gallinarum* and killed *Salmonella pullorum* vaccine to protect layer chicken from infection with *Salmonella Pullorum*.by assessment the bacterial shedding, morbidity and mortality and histological changes in internal organs.

3. METHODOLOGY

3.1. Ethical Approval

Ethical approval was granted according to the local committee of care and use of the animal in research at the College of Veterinary Medicine, University of Baghdad (P-G/12196 at 10/11/2024) before starting this study.

3.2. Experimental design

Sixty-layer chickens (ISSA brown) at 100 days old were confirmed to be free of *S. pullorum* by culturing the fecal samples. The study was conducted from mid-December 2023 to January 2024 to evaluate the immunopathological study of an inactive *Salmonella pullorum* and 9R vaccine to protect layer's chicken from *Salmonella pullorum* infection. Orally challenged with *Salmonella pullorum* 0.5 ml (25×104 CFU/ml) was administered to all groups (OM988162.1). Following that, they are split up into three groups, each with 20 chickens: First group: received a 0.5 ml subcutaneous dose of the 9R S. gallinarum vaccine. The second group 0.5 ml of dead a subcutaneous dose of *S. pullorum* vaccine administered subcutaneous at 100 days of age. The third group considers as control negative. From the jugular vein, samples of blood were collected at 14, 15, 16, 17, and 18 weeks, the strain of vaccines used in the study includes *Salmonella gallinarum* 9R strain (Himmvac-Korea). *Salmonella pullorum* killed whole cell antigen was prepared according to the motive procedure (Duncan, 2024).

3.3. Histology

Briefly, the formalin fixed tissue sections were dehydrated by ethanol, cleared by xylene, infiltrated and embedded by paraffin, sectioned by microtome at 4-5 μm thickness and mounted on the slides to be stained later with the Hematoxylin and Eosin following the manufacturer instructions (Syrbio, Syria). The stained slides were visualized under an objective lens of 10x of light microscope (MEIJI, Japan), (Gharban *et al*., 2023; Hussen *et al*., 2024).

3.4. Statistical Analysis

The Statistical Analysis System (SAS) (2018) program was used to detect the effect of different factors on study parameters. The least significant difference (LSD) was used to compare the means (ANOVA/One-way) significantly (Gharban and Yousif, 2020).

4. RESULTS AND DISCUSSION

The observation of the results showed that all groups at 14, 18 and 19-weeks-old when utilizing the most probable number (MPN/g) method to count the amount of Salmonella in the chickens' feces, there were extremely noticeable differences at

level (P≤0.05) are presented in Table 1. At 14 weeks, the results of MPN/g not recorded any colony in all groups, whereas after challenge at 17 weeks the results of MPN/g revealed highest means at 18 weeks in G3 and G1 with lowest means relative to the G2. But the results of MPN/g show substantial drop counting number after infection (P≤0.05) at 19 weeks in all groups especially in G2 in comparison with G3 and G1, which have larger MPN/g means respectively.

Table 1. Salmonella in feces counted using the most probable number (MPN/g) approach

Groups	Period (week)		
	14	18	19
G1	0 ± 0 A	45.8 ± 8.3 B	20.7 ± 6.4 B
G2	0 ± 0 A	7.1 ± 2.4 C	1.2 ± 0.4 C
G3	0 ± 0 A	62.5 ± 14.7 A	35.6 ± 14.2 A
LSD		12.08	9.17
MPN/g Means ± Stander error			

Number of samples: 5 from each group. Significant differences between groups are indicated by capital letters (P≤0.05) on the means of the same column. LSD: less significant differences.

4.1. Morbidity and mortality

This study shows that the morbidity and mortality in groups after challenge with local isolate of *Salmonella Pullorum* at 17 weeks, the results showed that were significant decrease (P≤ 0.05) in rates of morbidity and mortality specially in G2 vaccinated with killed vaccine followed by G1 that vaccinated with 9R, while control groups G3 showed a high significant increase (P≤0.05) in morbidity and mortality rates (Table 2).

Table 2. Development of clinical signs and mortalities during 18 weeks post challenge with local *S. pullorum* isolate at 17 weeks of age

Groups	Index [% (No.)]	
	Morbidity	Mortality
G1	40% (4/10) b	30% (3/10) b
G2	20% (2/10) c	0% (0/10) c
G3	100% (10/10) a	50% (5/10) a

Number of chickens for each group: 10. = Number of chickens showing clinical signs or death. G1: vaccinated with 9R *Salmonella gallinarum* live vaccine. G2: vaccinated with killed *Salmonella Pullorum* vaccine. G3: control negative not vaccinated. All groups challenge with local strain of *Salmonella pullorum* (25×104 cfu/ml) at 17 weeks. The small letters appear on the means of the same column refer to significant differences among treatment means at (P≤0.05).

The histopathological results post-challenge with *Salmonella pullorum* (25×104 cfu/ml) at 17 weeks of the liver in G1 group showed multiple MNCs infiltration and mild sinusoidal congestion with prominence of Kupffer cells. In G2 group, showed moderate MNCs infiltration with heterophils were reported in portal region accompanied with venous dilation. G3 group, see in other section revealed central vein congestion and dilation with perivascular leukocytic aggregation accompanied with necrosis of adjacent hepatic cells (Figure 1).

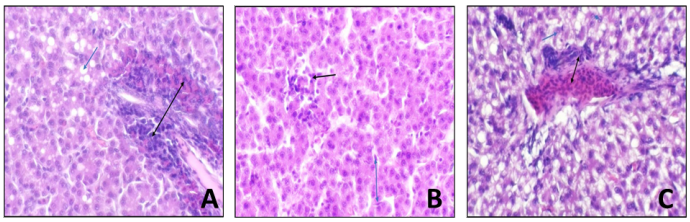

Figure 1. (A) Liver section G1 showed marked aggregation of lymphocytes and heterophils around hepatic vessel (black arrow) with evidence of cytoplasmic fat droplets in adjuvant hepatocytes (blue arrow) (H and E stain, 40X). (B) Liver section G2 showed Kupffer cells proliferation (blue arrow) and small leukocytic aggregation (black arrow) (H and E stain, 40X). (C) Liver section G3 revealed C.V congestion and dilation with perivascular lymphocytic aggregation (black arrow) accompanied with necrosis of adjacent hepatic cells (blue arrow) (H and E stain, 40X).

The microscopic findings of the spleen in G1 group were characterized by lymphoid depletion. In G2 group, the findings showed moderate hyperplasia of lymphatic follicles with medial thickening of splenic vessels and mild red pulp congestion. G3 group, histopathological section in the spleen showed moderate necrotic finding of reticular tissue with sinus congestion and dilation (Figure 2).

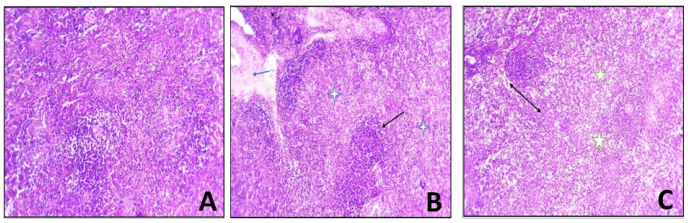

Figure 2. (A) Splenic histopathological finding of G1 characterized by lymphoid depletion (H and E stain, 10X). (B) Splenic section G2 showed moderate hyperplasia of lymphatic follicles (black arrow) with medial thickening of splenic vessels and mild red pulp congestion* (H and E stain, 10X). (C) Splenic section G3 revealed moderate necrotic finding of reticular tissue* with sinus congestion and dilation (black arrow) (H and E stain, 10X).

The microscopically findings of the cecum in G1 group showed the lamina propria is moderate expanded by lymphocytic inflammatory infiltrate also, revealed evident of GALT hyperplasia accompanied with moderate villous desquamation. In G2 group, revealed normal cecal folds with healthy villi (mild height villi), where see in other section marked obvious hyperplasia of lymphatic nodules with diffuse submucosal MNCs. G3 group, revealed moderate villus standing with remnant of atrophic lymphatic nodule also, showed abnormal irregular cecal folds with evidence of fecal mucosal ulceration (Figure 3).

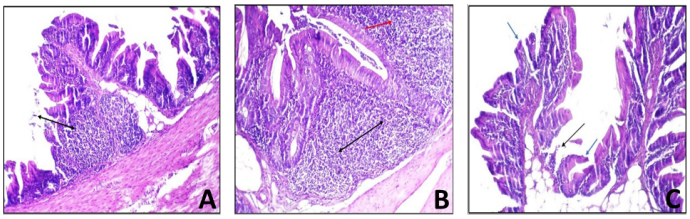

Figure 3. (A) Cecum section G1 showed evident of GALT hyperplasia accompanied with moderate villous desquamation (black arrow) (H and E stain, 10X). (B) Cecum section G2 revealed marked obvious hyperplasia of lymphatic nodules (black arrow) with diffuse submucosal MNCs (red arrow) (H and E stain, 40X). (C) Cecum section G3 revealed abnormal irregular cecal folds (blue arrow) with evidence of fecal mucosal ulceration (black arrow) (H and E stain, 10X).

The histopathological findings of the kidney in G1 group showed moderate tubular dilation of renal parenchyma with vacuolar degeneration of some tubules associated with mild interstitial MNCs infiltration and hemorrhage. In G2 group, exhibit prominence of basophilic cortical tubules with mild cellular swelling of major renal tubules. G3 group, focal tubular dilation with flat epithelial lining with interstitial hemorrhage accompanied with focal MNCs infiltration mainly between degenerated tubules (Figure 4).

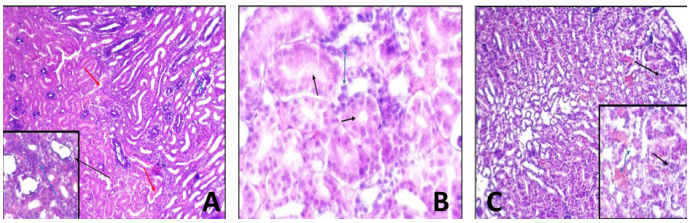

Figure 4. (A) Kidney section G1 showed moderate tubular dilation of renal parenchyma with vacuolar (black arrow) degeneration of some tubules associated with mild interstitial MNCs infiltration (blue arrow) and hemorrhage (red arrow) (H and E stain, 40X and 10X). (B) Kidney section G2 showed prominence of basophilic cortical tubules (black arrow) with mild cellular swelling of major renal tubules (blue arrow) (H and E stain, 40X). (C) Kidney of G3 group post-challenge revealed focal tubular dilation with flat epithelial lining with interstitial hemorrhage accompanied (blue arrow) with focal MNCs infiltration mainly between degenerated tubules (black arrow) (H and E stain, 10X and40X)

4.2. Discussion

The results of the most probable number showed in all groups no Salmonella shedding in their feces at 14 weeks between vaccinated and unvaccinated groups. These findings are in agreement with a previous study (Gole *et al.*, 2014). Whom recorded results of the culture method indicated that the age of 14 weeks, the prevalence of Salmonella in feces was not recorded in any colony in all groups. Differences in Salmonella counts between groups were evident by 18 weeks post-challenge with *Salmonella pullorum*. However, G1 vaccinated with the 9R S. gallinarum live vaccine had a moderate reduction in salmonella shedding; this is consistent with Al-Zuhariy (2023) who reported live Salmonella vaccines have been shown to prioritize cell-mediated immunity, which may not significantly reduce fecal shedding. On other hand the count of Salmonella in the controls G3 was the highest, this is consistent with the researcher (Ishola, 2009; Yue *et al.*, 2025) who recorded that Challenge dose significantly affects Salmonella shedding in experimentally infected chickens, the lack of prior immunity allowed *Salmonella pullorum* to multiply unchecked in the intestinal tract, resulting in increased shedding. Foremother, the killed vaccine with the best protection and least Salmonella shedding, even in the post-challenge- period, likely resulting from a more robust immune response and these results are consistent with previous studies (Al–Rubaye *et al.*, 2023, Hasan *et al.*, 2023) who recorded that killed Salmonella vaccine provided greater protection against Salmonella colonization in laying hens compared to live vaccines. chicken immunized showed lowest morbidity the clinical symptoms were slight and temporary following challenge in immunized chicken with killed *salmonella pullorum* vaccine compared with those in G1 9R live vaccine and the control group these results are in agreement with, whom recorded that the killed *salmonella pullorum* vaccine can offer efficient protection against acute systemic Pullorum diseased infection through stimulating both humoral and cell-mediated immune responses. Morbidity rates were significantly lower in the vaccinated groups than in the controls, with the G2 killed vaccine having the lowest morbidity. This also seems to support by Eeckhaut *et al.* (2024) who proposal that killed vaccines have the potential to elicit stronger and more durable immune responses than other types of vaccines. The unvaccinated group G3 showed the highest levels of morbidity and mortality of all, demonstrating the susceptibility of unvaccinated chickens to Salmonella infection (Alsadwi, 2024), they proved that lack of pre-existing immunity in the absence of vaccination, birds lack specific antibodies or memory cells to mount an effective defense against *S. pullorum*. Unregulated immune activation causes severe inflammation, further tissue damage and several lesions. On the other side, G1 showed limited protection against morbidity and mortality in chickens challenged with virulent *S. pullorum* (Lee *et al.*, 2007). *Salmonella pullorum* caused bacteremia and colonized to varying degrees in the liver, spleen, kidney, and intestine of chickens, according to results from the oral inoculation route (Shivaprasad & Barrow, 2008). In fact, all organs showed levels of infection in this investigation, which is similar to the results of a previous study by Noomi (2018) who examines the pathology and colonization of internal organs in broiler chickens after experimental oral infection with *Salmonella gallinarum*, highlighting differences in pathological changes. On the other hand, the results of histopathological changes post-challenge with *Salmonella pullorum* of where in G1 showed in the liver marked aggregation of leukocytes around hepatic vessels mainly consist of lymphocytes and heterophils with evidence of cytoplasmic fat droplets in adjuvant hepatocytes, in G2 showed Kupffer cells proliferation and small leukocytic aggregation. Moreover, the control group revealed focal MNCs infiltration, forming granulomatous lesion with sinusoidal dilation and congestion, these finding was also recorded (Ismail & Garo, 2009; Shahinuzzaman *et al.*, 2011; Purwanti *et al.*, 2019; Rahman *et al.*, 2019; Shchebentovska *et al.*, 2021). Previous reports have

documented similar degenerative, necrotic, and infiltrative lesions, with results showing that while the live vaccine primed the immune system, it did not completely prevent hepatic stress or inflammation. Similar liver changes were documented in *Salmonella pullorum*-infected birds, with the vaccinated groups showing milder changes compared to controls. They provided better protection with minimal liver damage, reflecting their role in eliciting a balanced immune response.

While the microscopic changes in the spleen are characterized in the first group by lymphoid depletion, also in G2, moderate hyperplasia of lymphatic follicles with medial thickening of splenic vessels; besides, in the G3 group, moderate necrotic findings of reticular tissue with sinus congestion and dilation have been reported by various studies that recorded similar changes in the spleen have been reported in poultry exposed to bacterial challenges, emphasizing the role of vaccination in modulating splenic responses (Prasanna *et al.*, 2001; Garcia *et al.*, 2010; Al-Juburi and Al-Sammarraae, 2022; Rani *et al.*, 2022). Live vaccines often induce stronger but less sustained immune responses, while killed vaccines provide more measured and longer-lasting immunity.

At the same time, the pathological changes that occurred in the intestine post-challenge, where in G1, the lamina propria, are moderately expanded by lymphocytic inflammatory infiltrates and GALT hyperplasia accompanied by moderate villous desquamation. Also, in G2, obvious hyperplasia of lymphatic nodules with diffuse submucosal MNCs. either in G3 revealed abnormal irregular cecal folds with evidence of fecal mucosal ulceration. All these lesions were also observed by several studies that reported the mild villous desquamation indicates the vaccine's live component provoked a moderate inflammatory reaction but not excessive tissue damage, which that killed indicates robust immune activation while maintaining intestinal structure (Fasina *et al.*, 2010; Abdulridha & Ibrahim. 2018; Al-Zuhariy, 2023; Hamad *et al.*, 2024). The moderate increase in villus height further signifies improved nutrient absorption and recovery post-challenge, while severe epithelial and mucosal damage due to an uncontrolled Salmonella infection. While in the kidney the microscopically changes characterized in G1 by moderate tubular dilation of renal parenchyma with vacuolar degeneration of some tubules associated with mild interstitial MNCs infiltration and hemorrhage also in G2, prominence of basophilic cortical tubules with mild cellular swelling of major renal tubules where G3, evidence of necrotic findings were recorded in both tubular and glomerular tissue with evident glomerular tuft atrophy Similar degenerative and infiltrative changes have been described in the kidneys of birds affected by *S. pullorum* (Shivaprasad, 2000; Deshmukh *et al.*, 2007; Rahman *et al.*, 2011; Saha *et al.*, 2012; Ali *et al.*, 2024); in which, the vacuolar degeneration and tubular dilation indicate a moderate inflammatory response to challenge, the live vaccine elicited a protective immune response but was not fully effective in preventing kidney damage. The milder pathological changes in this group reflect the stronger protective effect of the killed vaccine in mitigating kidney damage after challenge. The extensive necrotic findings in both tubular and glomerular tissue highlight the uncontrolled progression of Salmonella infection in the absence of vaccination.

5. CONCLUSION

This study demonstrates that immunization with the killed *Salmonella pullorum* vaccine provides superior protection to layer hens against *Salmonella pullorum* infection compared to the 9R live vaccine. Our findings indicate that the killed vaccine significantly reduces pathological changes, evidenced by moderate mononuclear cell (MNC) infiltration with heterophils and minimal impact on liver histology. In contrast, the 9R vaccine showed more pronounced histopathological alterations, including multiple MNC infiltration and mild sinusoidal congestion. Importantly, the killed vaccine group exhibited no bacterial shedding and maintained low morbidity without any recorded mortality, highlighting its effectiveness and safety as a preventive measure in poultry. These results underscore the potential of the killed *S. pullorum* vaccine in enhancing poultry health management and limiting the spread of infection, thereby contributing to more sustainable and productive poultry operations. Further studies are recommended to explore long-term immunity and scalability within commercial settings.

REFERENCES

Abdhalla, F., & Al-Gburi, N. M. (2023). Molecular Identification and Phylogenetic Analysis of Salmonella species Isolated from Diarrheal Children and Dogs in Baghdad Governorate, Iraq. *The Iraqi Journal of Veterinary Medicine, 47*(2), 50-58.

Abdulridha, R. N., & Ibrahim, O. M. S. (2018). Activity of bacterial antibiotics against some pathogenic bacteria isolated from calves diarrhoea in Baghdad (Part I). *Iraqi Journal of Agricultural Sciences, 49*(5).

Ali, A. J., Nijres, A. T., & Faraj, R. A. (2024). Detection of Mycoplasma gallisepticum and Mycoplasma synoviae in Fertile Eggs by ELISA and Real-Time PCR. *The Iraqi Journal of Veterinary Medicine, 48*(2), 98-104.

Al-Juburi, L. I., & Al-Sammarraae, I. A. (2022). The protective role of Salmonella typhimurium-whole sonicated killed antigen and Syzygium aromaticum extract on the histopathological changes against its infection in rabbits. *The Iraqi Journal of Veterinary Medicine, 46*(2), 12-19.

Al–Rubaye, M. S., & Al-doori, A. A. (2023). Detection of some virulence, antibacterial resistance genes for Salmonella isolated from dogs in Baghdad city. *Iraqi Journal of Agricultural Sciences, 54*(3), 741-747.

Alsadwi, A. M., Ibrahim, M. M., Shah, K. N., Cannon, C. L., Byrd, J. A., Caldwell, D., & Bailey, C. A. (2024). In Vitro Efficacy of Silver Carbene Complexes, SCC1 and SCC22, Against Some Enteric Animal Pathogens. *The Iraqi Journal of Veterinary Medicine, 48*(1), 1-8.

Al-Zuhariy, M. T. (2023). Evalution of the best vaccinal routes against newcastle in the production stage of laying hens. *Iraqi Journal of Agricultural Sciences, 54*(3), 748-754.

Al-Zuhariy, M. T. (2023). Protection activity of t cell lymphokines against infectious bursal disease in layer pullets. *Iraqi Journal of Agricultural Sciences, 54*(4), 1118-1124.

Barrow, P. A. (2007). Salmonella infections: immune and non-immune protection with vaccines. *Avian pathology, 36*(1), 1-13.

Barrow, P. A., & Wallis, T. S. (2000). *Vaccination against Salmonella infections in food animals: rationale, theoretical basis and practical application.*

Buck, J. D., Immerseel, F. V., Haesebrouck, F., & Ducatelle, R. (2004). Effect of type 1 fimbriae of Salmonella enterica serotype Enteritidis on bacteraemia and reproductive tract infection in laying hens. *Avian Pathology, 33*(3), 314-320.

Deshmukh, S., Asrani, R. K., Ledoux, D. R., Rottinghaus, G. E., Bermudez, A. J., & Gupta, V. K. (2007). Pathologic changes in extrahepatic organs and agglutinin response to *Salmonella Gallinarum* infection in Japanese quail fed Fusarium verticillioides culture material containing known levels of fumonisin B1. *Avian Diseases, 51*(3), 705-712.

Duncan, I. J. (2024). The domestic fowl. *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals*, 739-761.

Eeckhaut, V., Van Rysselberghe, N., Verbanck, S., Ducatelle, R., & Van Immerseel, F. (2024). Oral vaccination of young broilers with a live Salmonella Typhimurium vaccine reduces caecal and internal organ colonization following a Salmonella Infantis challenge in a seeder-bird model. *Avian Pathology, 53*(6), 492-498.

Eriksson, H., Söderlund, R., Ernholm, L., Melin, L., & Jansson, D. S. (2018). Diagnostics, epidemiological observations and genomic subtyping in an outbreak of pullorum disease in non-commercial chickens. *Veterinary Microbiology, 217*, 47-52.

Fasina, Y. O., Hoerr, F. J., McKee, S. R., & Conner, D. E. (2010). Influence of Salmonella enterica serovar Typhimurium infection on intestinal goblet cells and villous morphology in broiler chicks. *Avian Diseases, 54*(2), 841-847.

Freitas Neto, O. C., Arroyave, W., Alessi, A. C., Fagliari, J. J., & Berchieri, A. (2007). Infection of commercial laying hens with *Salmonella Gallinarum*: clinical, anatomopathological and haematological studies. *Brazilian Journal of Poultry Science, 9*, 133-141.

Garcia, K. O., Santana, A. M., Freitas Neto, O. C., & Simplício, K. M., Alessi, A. C., Berchieri Júnior, Â., & Fagliari, J. J. (2010). *Experimental infection of commercial layers using a Salmonella enterica sorovar Gallinarum strain: blood serum components and histopathological changes.*

Gast, R. K., & Porter Jr, R. E. (2020). Salmonella infections. *Diseases of poultry*, 717-753.

Gharban, H. A., & Yousif, A. A. (2020). Serological and molecular phylogenetic detection of Coxiella burnetii in lactating cows, Iraq. *The Iraqi Journal of Veterinary Medicine, 44*(E0), 42-50.

Gharban, H. A., Al-Shaeli, S. J., & Hussen, T. J. (2023). Molecular genotyping, histopathological and immunohistochemical studies of bovine papillomatosis. *Open Veterinary Journal, 13*(1), 26-41.

Gole, V. C., Caraguel, C. G., Sexton, M., Fowler, C., & Chousalkar, K. K. (2014). Shedding of Salmonella in single age caged commercial layer flock at an early stage of lay. *International journal of food microbiology, 189*, 61-66.

Guo, R., Li, Z., Zhou, X., Huang, C., Hu, Y., Geng, S., & Jiao, X. (2019). Induction of arthritis in chickens by infection with novel virulent *Salmonella Pullorum* strains. *Veterinary microbiology, 228*, 165-172.

Hamad, M. H., Al–Hayani, W. K. A., & Hussein, F. M. (2024). Effects of live body weight and leptin concentration on iraqi chickens fertility and hatchability. *Iraqi Journal of Agricultural Sciences, 55*(3), 1221-1232.

Hasan, T. O., Lafta, I. J., Ahmed, E. A., & Jassam, S. A. (2023). Application of RAPD-PCR and phylogenetic analysis for accurate characterization of Salmonella spp. isolated from chicken and their feed and drinking water. *The Iraqi Journal of Veterinary Medicine, 47*(1), 11-20.

Hussen, T. J., Al-Shaeli, S. J. J., Al-Mahna, B. H. R., & Gharban, H. A. J. (2024). Biochemical and histological effects of long-term administration of estrogen on female mice. *Adv. Anim. Vet. Sci, 12*(8), 1563-1572.

Ishola, O. O. (2009). Effects of challenge dose on faecal shedding of Salmonella enteritidis in experimental infected chickens. *African Journal of Biotechnology, 8*(7).

Islam, M., Aziz, H., Bacha, Q., Ullah, S., & Ullah, I. (2025). Isolation and identification of salmonella spp from poultry meat samples. *The Research of Medical Science Review, 3*(1), 1015-1020.

Ismail, H. K., & Garo, H. M. (2009). *Pathological changes in livers of broiler chickens with unabsorbed yolk sac.*

Kabeta, T., Tolosa, T., Duchateau, L., Van Immerseel, F., & Antonissen, G. (2024). Prevalence and serotype of poultry salmonellosis in Africa: A systematic review and meta-analysis. *Avian Pathology, 53*(5), 325-349.

Kang, X., Yang, Y., Meng, C., Wang, X., Liu, B., Geng, S., & Pan, Z. (2022). Safety and protective efficacy of *Salmonella Pullorum* spiC and rfaH deletion rough mutant as a live attenuated DIVA vaccine candidate. *Poultry Science, 101*(3), 101655.

Lee, Y. J., & Kang, M. S. (2007). Protective efficacy of live *Salmonella gallinarum* 9R vaccine in commercial layer flocks. *Avian Pathology, 36*(6), 495-498.

Noomi, B. S. (2018). Comparative diagnostic study of avian Salmonellosis in Salahaldeen province. *Iraqi Journal of Agricultural Sciences, 49*(5).

Prasanna, K., Somvanshi, R., & Paliwal, O. P. (2001). Experimental

fowl typhoid and Pullorum disease infection in chicken: Histopathological and ultrastructural studies on small intestine and liver. *Indian Journal of Veterinary Pathology, 25*(1 and 2), 18-20.

Purwanti, S., Agustina, L., Syamsu, J. A., & Putra, R. D. (2019, March). Histology of the liver and small intestine broiler using phytobiotic in the ration infected *Salmonella pullorum*. In *IOP Conference Series: Earth and Environmental Sciencem, 247*(1), 012054.

Rahman, M. M., Alam, J., Khan, M. A. H. N. A., & Haider, M. G. (2019). Pathology of Pullorum Disease and Molecular Characterization of Its Pthogen. *Annals of Bangladesh Agriculture, 23*(1), 25-35.

Rahman, M. R., Shahinuzzaman, A. B. M., Saha, A. K., Sufian, M. A., Rahman, M. H., & Hossain, M. M. (2011). Prevalence of Salmonella infection in naturally infected layer of birds in Bangladesh. *Bangladesh Veterinarian, 28*(1), 8-18.

Rani, S., Alam, K. J., Saha, S. S., Rahman, M. M., & Alam, M. S. (2022). Seroprevalence, identification, and pathology of salmonellosis in selected poultry farms at Barishal District of Bangladesh. *Turkish Journal of Agriculture-Food Science and Technology, 10*(6), 1158-1164.

Revolledo, L. (2018). Vaccines and vaccination against fowl typhoid and pullorum disease: An overview and approaches in developing countries. *Journal of Applied Poultry Research, 27*(3), 279-291.

Saha, A. K., Sufian, M. A., Hossain, M. I., & Hossain, M. M. (2012). Salmonellosis in layer chickens: pathological features and isolation of bacteria from ovaries and inner content of laid eggs. *Journal of the Bangladesh Agricultural University, 10*(1), 61-67.

Saleem, G., Ramzaan, R., Khattak, F., and Akhtar, R. (2016). Effects of acetic acid supplementation in broiler chickens orallychallenged with *Salmonella Pullorum. Turkish Journal of Veterinary and Animal Sciences, 40*(4), 434-443.

Shahinuzzaman, A. B. M., Saha, A. K., Mazumder, A. C., Das, S., Sufian, M. A., Baki, M. A., & Hossain, M. M. (2011). Sequential pathological and immunohistochemical study of Pullorum Disease in experimentally infected chicks. *International Journal of Poultry Science, 10*(5), 404-410.

Shchebentovska, O., Kostynuk, A., Zaika, S., Kovalova, L., Yevtukh, L., & Holubtsova, M. (2021). Pathomorphological changes in the organs of chickens infected spontaneously by the species *Salmonella pullorum* on private farms in chernivtsi region. *Regulatory Mechanisms in Biosystems, 12*(4), 614-619.

Shen, X., Zhang, A., Gu, J., Zhao, R., Pan, X., Dai, Y., & Zhang, D. (2022). Evaluating *Salmonella pullorum* dissemination and shedding patterns and antibody production in infected chickens. *BMC Veterinary Research, 18*(1), 240.

Shivaprasad, H. L. (2000). Fowl typhoid and pullorum disease. Revue scientifique et technique (International Office of Epizootics), 19(2), 405-424.

Shivaprasad, H. L., and Barrow, P. A. (2008). Pullorum disease and fowl typhoid. *Diseases of poultry*, 620-634.

Tian QiuFeng, T. Q., Zhou Wei, Z. W., Si Wei, S. W., Yi Fei, Y. F., Hua Xin, H. X., Yue Min, Y. M., & Yu ShenYe, Y. S. (2018). *Construction of Salmonella Pullorum ghost by co-expression of lysis gene E and the antimicrobial peptide SMAP29 and evaluation of its immune efficacy in specific-pathogen-free chicks.*

Vaid, R. K., Thakur, Z., Anand, T., Kumar, S., & Tripathi, B. N. (2021). Comparative genome analysis of Salmonella enterica serovar Gallinarum biovars Pullorum and Gallinarum decodes strain specific genes. *PLoS One, 16*(8), e0255612.

Valentina, M. G., Natalia, M. J., Ligia, S. L., & Gomez, A. P. (2022). *Microbiological and molecular techniques for the identification of Salmonella sp in the poultry industry: a systematic scoping review.*

Van Immerseel, F., Methner, U., Rychlik, I., Nagy, B., Velge, P., Martin, G., & Barrow, P. A. (2005). Vaccination and early protection against non-host-specific Salmonella serotypes in poultry: exploitation of innate immunity and microbial activity. Epidemiology and Infection, 133(6), 959-978.

Yue, W. Y., & Groves, P. J. (2025). Age of challenge is important in Salmonella Enteritidis studies in pullets and hens: a systematic review. *Avian Pathology, 54*(2), 159-167.