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Research Article

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Integrated Clinicopathological and Immunological Responses to Experimental *Salmonella* Enteritidis Infection in Broiler Chickens

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ABSTRACT

Background: Salmonellosis remains a major challenge in poultry production due to economic impact and zoonotic significance. In broiler chickens, *Salmonella* Enteritidis impairs intestinal function and reduces growth performance. However, integrated evaluations of clinical, pathological, hematological, and immunological responses are limited.

Aims: This study aimed for characterization of broiler clinicopathological responses following experimental S. Enteritidis infection under conditions approximating natural exposure.

Methods: 20 clinically healthy, *Salmonella*-free Cobb 500 broiler chickens were obtained as day-old chicks (DOC) from JAPFA Comfeed, Indonesia. Chickens were randomly allocated into infected (n=10) and uninfected control (n=10) groups. Infected group chickens were orally inoculated with S. Enteritidis ATCC 13076. Birds were observed for four weeks post-inoculation before clinical, hematological, histopathological, histomorphometry, and immunohistochemical evaluations.

Results: Infected chickens developed enteric clinical signs including diarrhea, lethargy, soiling, and reduced feeding. Body weight gain was significantly lower in infected birds (ΔI=0.64kg ± 0.24 vs ΔC= 1.07kg ± 0.37; p=<0.001). Histomorphometry analysis showed statistically significant reduction in ileal villus height (p=<0.001) and cecal crypt depth (p=<0.001). Histopathology demonstrated mild epithelial desquamation and goblet cell hyperplasia with minimal hepatic alterations. The complete blood counts indicated leukocytosis, primarily heterocytosis, with no significant differences in erythrocyte indices. Immunohistochemistry may reflect a potential increase in ileal IL-2 expression (p=<0.001) and no changes to TNF-α expression (p>0.05).

Conclusion: Experimental S. Enteritidis infection induced systemic leukocytic response and the results may reflect enhanced local IL-2 expression that resulted in localized intestinal injury and altered growth performance, indicating coordinated clinicopathological adaptation largely confined to the gastrointestinal tract.

INTRODUCTION

Increasing global demand for poultry as an affordable and accessible animal protein has intensified broiler production systems worldwide (Wessels et al., 2021). Although this expansion supports food security and economic growth, it is frequently associated with greater disease pressure from high stocking density, environmental stress, and variable biosecurity implementation (Adji et al., 2019; Wessels et al., 2021; Galán-Relaño et al., 2023). Salmonellosis is among the most important poultry infections, with adverse effects

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on animal health and productivity alongside significant zoonotic ramifications: non-typhoidal *Salmonella* is a major cause of enteritis globally, with poultry as primary sources of human exposure (European Food Safety Authority, 2021). Among circulating serovars, *Salmonella* Enteritidis incites concern with its efficient intestinal colonization and rapid horizontal spread (Qayum et al., 2025; Williams et al., 2025). As a result, *S.* Enteritidis control is a priority in poultry health and food safety programs.

In broiler chickens, *Salmonella* infection ranges from asymptomatic intestinal colonization to clinical enteritis and, less commonly, systemic dissemination. Affected birds may exhibit low appetite, diarrhea, disheveled appearances, and growth and activity impairment (Kaiser et al., 2000; Shaji et al., 2023). Infection begins with pathogen ingestion, then adhesion and invasion of intestinal epithelial cells, followed by immune response activation. The ileum and cecum serve as major sites of bacterial persistence and host–pathogen interaction and infection may induce villus shortening, epithelial desquamation, crypt hyperplasia, and altered goblet cell activity culminating in reduced absorptive capacity, contributing to decreased weight gain and production losses (Wang et al., 2016; Ijaz et al., 2021; Ayuti et al., 2024).

Host immunity is critical to infection outcomes. Early defense and recovery prospects against *Salmonella* appear primarily defined by innate immune responses, particularly heterophils (Ijaz et al., 2021). Adaptive immune response is documented though not well-characterized as poultry cytokine research is in its infancy, especially in non-neonatal birds (Rohde et al., 2018; Milby-Blackledge et al., 2024). Specific cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-2 (IL-2) have been observed acting as mediators in poultry immune systems, with TNF-α promoting inflammatory amplification and leukocyte recruitment while IL-2 supports T-cell proliferation and targeted bacterial elimination (Withanage et al., 2005; Susta et al., 2015; Lu et al., 2022).

Despite advances in avian immunology, integrated multi-parameter characterizations of poultry salmonellosis are limited. Previous studies focus on rare case studies (Pshenichnaya et al., 2023; Devi et al., 2025) pathogen detection for epidemiological surveillance (Khoerunnisa et al., 2022; Nathania et al., 2022; Larasati et al., 2025) and development of preventive strategies (Wardhana et al., 2021; Srimaryanto et al., 2025). Although these approaches provide valuable information for disease control, they assess disease aspects in isolation and limit observation of coordinated host infection responses. Consequently, the relationships between clinical manifestations, tissue pathology, hematology, and immune activation remain insufficiently understood under conditions approximating natural exposure.

This study investigates multiple clinicopathological responses from broilers experimentally infected with *S.* Enteritidis under near-natural conditions. Clinical findings, changes in body weight, histopathological and histomorphometry alterations, hematological profiles, and immunohistochemical expression of TNF-α and IL-2 were evaluated simultaneously. We hypothesized based on past literature that infected broilers would exhibit reduced growth, intestinal remodeling, altered blood compositions, and increased local cytokine expression compared with uninfected controls.

MATERIALS AND METHODS

Research Preparation

This study used an *in vivo* experimental design to evaluate clinicopathological responses of broiler chickens following infection with *Salmonella* Enteritidis. The experiment was conducted from November 2025 to April 2026 in Dramaga District, Bogor Regency, West Java, Indonesia. Sample processing and laboratory analyses were performed at the Pathology Division Laboratories in the School of Veterinary Medicine and Biomedical Sciences, IPB University, and the iRatCo Laboratory in Dramaga.

Ethical Approval

All procedures involving animals were conducted in accordance with institutional animal welfare and biosafety regulations, approved by the Animal Ethics Committee School of Veterinary Medicine and Biomedical Sciences at IPB University under the permit number 407/KEH/SKE/XI/2025.

Experimental Design

Twenty Cobb 500 broiler chickens were obtained as day-old chicks from JAPFA Comfeed, Indonesia and reared for six weeks under standardized husbandry conditions with ad libitum access to feed and water. All birds were confirmed clinically healthy and *Salmonella*-free, before random allocation into infected (n = 10) and control (n=10) groups, and kept separate under identical environmental and biosecurity conditions.

Salmonella Enteritidis ATCC 13076 (American Type Culture Collection, USA) was freshly prepared into a bacterial suspension at a final concentration of 1×10^6 CFU/bird and administered orally through drinking water only to birds within the infected group, simulating

natural exposure. Water intake was monitored to ensure exposure consistency. Birds were observed for four weeks post-infection (wpi) before clinical, hematological, histopathological, histomorphometry, and immunohistochemical evaluations.

Clinical and Biometric Evaluation

Clinical observations were performed daily, then twice daily during active infection. Observed variables for disease progression included behavior, feed and water consumption, plumage as well as comb and wattle condition, fecal consistency, and locomotor activity. Body weight was recorded weekly to evaluate growth performance, then a final time pre-euthanasia.

Blood Collection and Hematological Analysis

Blood samples were collected aseptically from the brachial vein into EDTA tubes before euthanasia. Hematological evaluation used an automated veterinary hematology analyzer to obtain standard complete blood count (CBC) parameters.

Necropsy and Tissue Collection

Birds were humanely euthanized through jugular exsanguination according to institutional animal welfare guidelines. Ileum, cecum, and liver samples were collected immediately and fixed in 10% neutral buffered formalin (NBF) for histopathological and immunohistochemical examination.

Histopathological and Histomorphometry Examination

Tissue samples were routinely processed, paraffin-embedded, sectioned at 4–5 µm, stained with hematoxylin-eosin (HE) and assessed microscopically. Histomorphometry analysis employed ImageJ software (National Institutes of Health, USA).

Intestinal and Liver Measurements

For each small intestine specimen, ten intact and well-oriented structures of villus height, crypt depth, and muscularis propria thickness were evaluated in the ileum and cecum. Qualitative assessment included epithelial desquamation, inflammatory infiltration, goblet cell hyperplasia, crypt abnormalities, hepatic lipidosis, and tissue architectural alterations. Lesions were described as mild, moderate, or severe. Quantitative liver evaluation included hepatocyte nuclear area and eosin staining intensity.

Immunohistochemical Evaluation

Immunohistochemistry (IHC) was performed using anti-TNF-α and anti-IL-2 primary antibodies (Santa Cruz Biotechnology, USA). Standard antigen retrieval, blocking, antibody incubation, HRP detection, and DAB visualization procedures were applied. Positive results for the cytokines will stain shades of brown as opposed to blue. Cytokine expression was recorded semi-quantitatively through ImageJ image processing as Mean Grey Values and percentage values of positive staining area. Due to resource limitations, IHC analysis was conducted on only three ileum samples per group.

Statistical Analysis

Quantitative data from histomorphometry, hematology, immunohistochemistry, and body weight were compared between groups. Normality and homogeneity were assessed using the Shapiro–Wilk and Bartlett's tests respectively. Parametric data was analyzed using independent Student's *t*-test or Welch's *t*-test when variance assumptions were not met, whereas non-parametric data was analyzed using the Mann–Whitney *U* test. Body weight changes were evaluated using repeated measures analysis and linear regression. Statistical significance was set at $p < 0.05$. Qualitative findings were summarized descriptively.

RESULTS AND DISCUSSION

Clinical Observations and Body Weight

Broiler chickens experimentally infected with *Salmonella* Enteritidis developed clinical signs consistent with enteric salmonellosis and differed clearly from controls. Following inoculation, infected birds exhibited lethargy, reduced feed intake, increased defecation frequency, feather soiling, and deterioration of plumage condition, whereas no such clinically observable abnormalities occurred in controls. Clinical signs appeared approximately 1 wpi, were most pronounced during the early phase of infection, and gradually declined thereafter. This pattern is consistent with previous reports showing that *S.* Enteritidis infection in broilers commonly presents as an acute enteric disease followed by partial clinical recovery despite possible persistence of intestinal colonization (Shaji et al., 2023; Yue & Groves, 2024). The absence of severe systemic manifestations such as respiratory or neurological issues suggests that infection remained predominantly localized to the gastrointestinal tract, typical to non-invasive *Salmonella* Enteritidis serovars (Kurtz et al., 2017).

Body weight measurements further demonstrated the physiological impact of infection. Although both groups gained weight throughout the study, infected chickens exhibited consistently lower growth rates and greater variability with growth stagnation becoming more apparent during later observation periods (Figure 1). The final body weight gain from the beginning to the end of the study was significantly lower in infected birds than in controls (ΔI=0.64kg ± 0.24 vs ΔC= 1.07kg ± 0.37), indicating reduced productive performance despite the absence of severe systemic disease.

The reduced growth observed in infected chickens is likely multifactorial. Decreased feed intake during illness may limit nutrient availability, while intestinal epithelial alterations can impair digestive and absorptive efficiency. In addition, activation of immune responses increases metabolic demand and diverts energy away from growth, whereas diarrhea may further contribute through nutrient and fluid loss. Similar reductions in body weight gain have been consistently reported in experimental poultry salmonellosis and are commonly associated with impaired intestinal integrity and increased immune-related energy expenditure (Hu et al., 2023; Shaji et al., 2023). Overall, these findings indicate that experimental *S.* Enteritidis infection induced a predominantly non-lethal enteric disease accompanied by measurable impairment of growth performance.

Changes in the Small Intestine and Liver

The ileum and cecum are the primary sites of *Salmonella* Enteritidis colonization in poultry and are important in disease development. Following oral exposure, bacterial interaction with the intestinal epithelium and local immune system may induce structural and functional changes that support bacterial persistence (Ijaz et al., 2021). Histomorphometry analysis showed a tendency toward reduced villus height in both the ileum (Figure 2A) and cecum of infected chickens, although these differences were not statistically significant in the latter. In contrast, crypt depth increased significantly in the cecum (Figure 2B) but not the ileum.

Qualitative examination revealed mild-to-moderate flattening and blunting in infected ileal villi with mild-to-moderate epithelial desquamation and increased inflammatory cell invasion into the lamina propria compared to the control (Figures 2C, 2D, 2E and 2F). These observations are indicative of significant villus atrophy, which alters epithelial turnover during enteric infection and impairs intestinal absorption, contributing to diarrhea and reduced growth performance (Napiórkowska-Baran et al., 2025). Mild ileal goblet cell hyperplasia appeared in five infected birds (Figure 2F), as goblet cells proliferate under infection to increase mucin production for physical and chemical defense (Duangnumsawang et al., 2021). Conversely, cecal crypt hyperplasia reflects

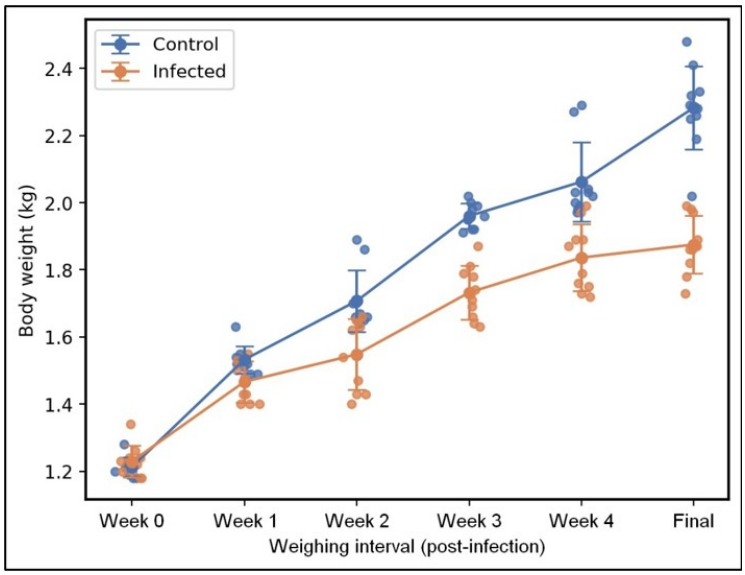


Figure 1. Line graph of body weight measurements post-inoculation. The bars depict standard deviation.

compensatory regenerative activity through enhanced epithelial proliferation to maintain mucosal integrity (Napiórkowska-Baran et al., 2025). While cecal villi remained largely intact, cecal crypt hyperplasia with mild crypt distortions and desquamation (Figures 2G, 2H and 2I) may imply complications with epithelial renewal from prolonged or chronic infection (Horton et al., 2026). This is consistent with the ileal damage and the moderate clinical symptoms of infected chickens at 4 wpi, while salmonellosis typically resolves under 2-3 wpi (Wigley, 2014). The more pronounced cecal crypt response and more pronounced ileal villus atrophy may indicate regional differences in tissue adaptation and possible limited mucosal remodeling: cecal crypt hyperplasia may have successfully compensated for villi damage, while ileal crypts may be returning to functionality without villi recovery. Clinical symptoms alleviating, though not resolving, at this stage corroborates this. Overall, it appears the ileum here was more impacted by infection than the cecum, possessing more examples of structural damage compared to the control. Previous literature, albeit outdated or using different models to adult chickens, suggest the inverse is usually true (Sivula et al., 2008; Lacroix-Lamandé et al., 2023).

Mild to minimal examples of intestinal alterations such as epithelial desquamation and goblet cell hyperplasia were observed in control chickens. Because conventional instead of specific pathogen-free (SPF) broilers were used for authenticity to normal rearing conditions, environmental exposure and normal biological variation cannot be completely excluded.

Hepatic evaluation showed minimal to nonexistent pathological change. Quantitative and qualitative analyses revealed preserved hepatic architecture

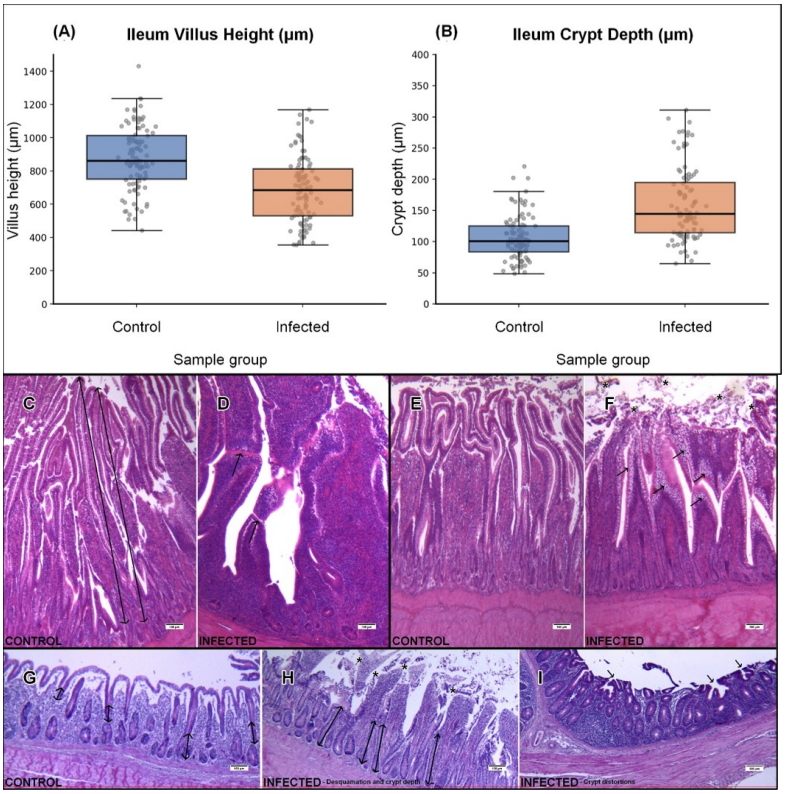


Figure 2. Box plots showing **(A)** ileal villus height and **(B)** cecal crypt depth. Representative H&E-stained sections showing **(C)** normal ileal villi in the control group (arrows) compared with **(D)** blunted ileal villi in the infected group (arrows), **(E)** normal ileal epithelium and goblet cells in the control group compared with **(F)** epithelial desquamation (asterisk) and goblet cell hyperplasia (arrowhead) in the infected group, and **(G)** normal cecal crypt depth (arrows) in the control group compared to **(H)** increased crypt depth (arrows), epithelial desquamation (asterisk), and **(I)** mild cecal crypt distortion (arrowhead) in the infected group.

without evidence of degeneration, inflammation, or lipid accumulation. These findings indicate once more that infection remained confined to the gastrointestinal tract without substantial systemic dissemination.

Overall, clinical and histomorphometry findings suggest that experimental *S.* Enteritidis infection was responsible for mild to moderate chronic enteric inflammation, characterized by adaptive epithelial regeneration and limited remodeling. The infection appeared non-systematic, as hepatic integrity was preserved and severe systemic clinical manifestations were absent.

Markers of Immune Activity through Hematology and Immunohistochemistry

Hematological and immunohistochemical (IHC) analyses were performed to evaluate systemic and local immune responses during *Salmonella* Enteritidis infection. Hematological parameters primarily reflected systemic leukocyte dynamics, whereas cytokine expression in ileal tissue represented local immune activity at the primary site of colonization.

One of the main findings was a significant increase of ileal IL-2 expression in infected chickens (Table 1), indicated by lower Mean Grey Values (Figure 3A) and greater DAB-positive coverage (Figure 3B) quantified from the IHC stains (Figures 3C and 3D). IL-2 is suspected to serve in T-cell proliferation and cellular immunity in poultry, and so this finding may reflect the presence of an adaptive immune response alongside the leukocytosis found in 9 of the infected birds (Figures 4A - 4F). No such substantial alterations were observed in erythrocyte parameters (Figures 4G - 4L), suggesting limited systemic effects beyond immune activation. Increased IL-2 expression together with the observed intestinal remodeling could also reflect coordinated mucosal adaptation during infection

Table 1. Representative values of IL-2 intensity (Mean Grey Value) and coverage (%).

Parameter	Group	Minimum	Median	Maximum	Mean	Interquartile Range (Q3-Q1)	p-value
IL-2 Intensity	Control	215.455	229.291	235.160	226.635	9.853	0.0005
	Infected	115.758	133.544	139.266	129.523	11.754	
IL-2 Coverage	Control	7.781	10.082	15.508	11.124	3.8635	0.0729
	Infected	47.63	54.605	100	67.412	26.185	

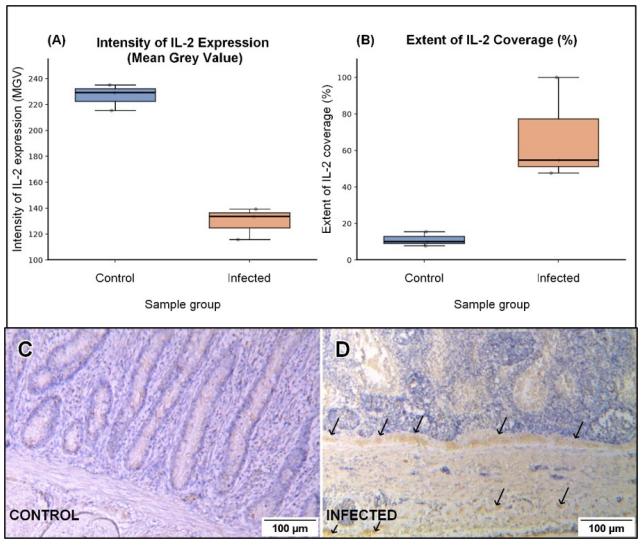


Figure 3. Box plots showing (A) ileal IL-2 immunohistochemical staining intensity and (B) IL-2-positive area coverage. Representative photomicrographs of ileal IL-2 immunohistochemical staining using DAB chromogen in the (C) control and (D) infected groups. Original magnification, ×10.

rather than direct cytokine-mediated tissue injury. However, lymphocyte counts specifically did not differ significantly between groups, and lymphocyte subsets were not analyzed within this study. Additionally, the sample size was extremely limited (n=3) and focused only on the ileum for IHC analysis. These limitations prevent definitive interpretations on the cytokine dynamics within this study without further investigation.

In contrast to the IL-2 results, TNF-α expression did not differ significantly between infected and control groups, even with heterocytosis. Given that TNF-α is primarily involved in early innate inflammatory responses (Kurtz et al., 2017), the absence of detectable differences may reflect temporal variation in cytokine expression, particularly because tissues were collected at 4 wpi when acute inflammation had likely subsided. Additionally, unlike mammalian neutrophils, heterophils do not rely on TNF-α-mediated oxidative burst (Ijaz et al., 2021). This interpretation could be consistent with the relatively mild histopathological lesions and limited inflammatory

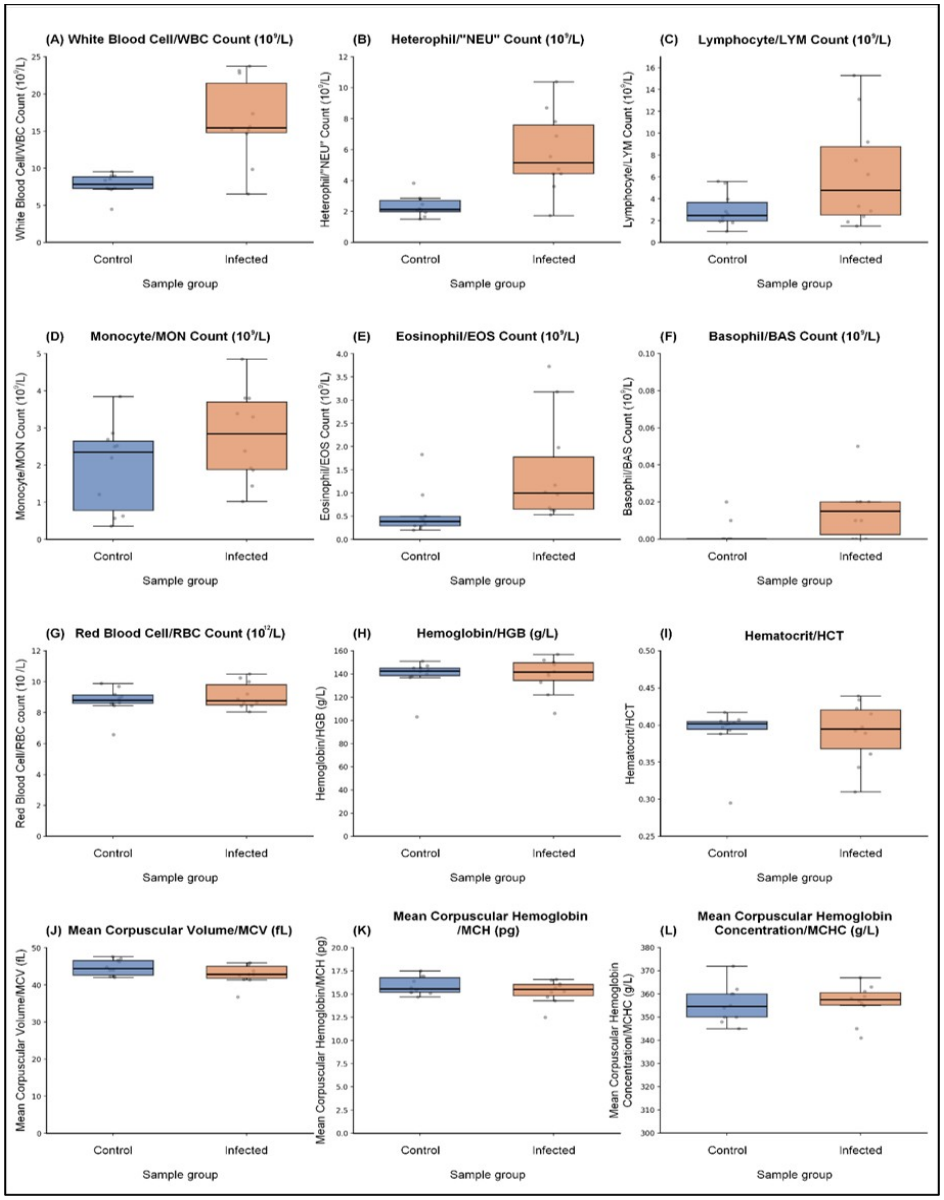


Figure 4. Box plots showing the hematological profiles: (A) - (F) depict leukocyte counts, whereas (G) - (L) depict the erythrocyte count and indices.

infiltration observed at the sampling endpoint, however this cannot be ascertained within this study alone due to the aforementioned sample limitations.

Other limitations of this study include the single endpoint evaluation, absence of bacterial re-isolation and the use of conventional non-SPF broilers that may have introduced background immune variation.

Overall, the findings indicate that *S.* Enteritidis infection induced measurable mucosal immune activation, characterized by leukocytic responses. Increased IL-2 expression and stable TNF-α activity was observed.

CONCLUSION

Salmonellosis in broiler chickens significantly affected multiple expected parameters: only the infected displayed clinical symptoms, histomorphometry indicates inflammatory damage, and hematology alongside IHC may reflect activity of the innate and adaptive immune system, though the latter requires further investigation for confirmation. The holistic model is a viable approach, though with parameters so intricately crosslinked further detailed research is required for the 'full picture'.

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AUTHORS CONTRIBUTION

The primary author of this work is T.R.P.S, alongside co-writer and head supervisor E.H. Significant contributors to the formation and execution of the methodology, sample collection, and data analysis were M.S, L.H and H.Y.

"The authors declare that there is no conflict of interest with the parties involved in this research".

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