

RESEARCH ARTICLE

Immunomodulatory Effects of Encapsulated IgY on Antibody Titers and Bursa Fabricius Integrity in Broilers Infected with *Salmonella Pullorum*

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Abstract

Passive immunization using immunoglobulin yolk (IgY) has attracted considerable attention as a prominent antibiotic alternative in poultry health management. However, its practical application is constrained by vulnerability to gastrointestinal degradation, limiting its immunoprotective potential against enteric pathogens like *Salmonella Pullorum*. To address this gap, this study investigated the protective efficacy of encapsulated IgY on bursa of Fabricius integrity and antibody responses in challenged Cobb 500 broilers. Under a completely randomized design, the chickens were allocated into a negative control group, a positive control group, and three treatment groups receiving encapsulated IgY at doses of 0.11, 0.22, and 0.34 mL/day, respectively. Encapsulated IgY administration was initiated at 8 days of age and continued for 14 consecutive days until the challenge infection was performed at 22 days of age via intramuscular injection into the pectoralis major muscle using a bacterial dose of 1.5×10^8 CFU. AGPT analysis demonstrated delayed antibody kinetics post-infection. Crucially, encapsulated IgY significantly countered *Salmonella*-induced lesions through distinct structural protections: minimizing follicular depletion (P1: 2.00 ± 0.37 vs K⁺: 2.80 ± 0.14), reducing lymphoid cell necrosis (P3: 1.36 ± 0.17 vs K⁺: 2.60 ± 0.20), and dose-dependently restoring plica diameter up to 243.02 ± 9.5 μ m (P1) and 224.78 ± 20 μ m (P2) at the highest dose. Although IgY technology shows immense therapeutic value, its encapsulated potential in veterinary medicine remains underexplored. This study provides a scientific rationale for utilizing encapsulated IgY as a physiological protective factor, advancing sustainable health management in livestock production, which directly aligns with current priority areas in veterinary research.

Keywords: Broiler Chickens, Bursa of Fabricius, Encapsulation, Immunoglobulin Yolk, *Salmonella Pullorum*

INTRODUCTION

Pullorum disease, caused by *Salmonella enterica* serovar Pullorum, continues to pose a major challenge to poultry production worldwide, particularly in developing regions [1]. This Gram-negative bacterium is highly host-specific and primarily infects young chicks, causing systemic illness characterized by anorexia, white diarrhea, dehydration, reduced growth, and high mortality rates [2]. One of the main organs targeted by *Salmonella Pullorum* infection is the Bursa of Fabricius, a lymphoid organ unique to avian species that is essential for the development and maturation of B lymphocytes [3]. Structural damage or atrophy of this

organ can impair humoral immune function and reduce the bird's resistance to secondary infections.

The growing concern regarding antimicrobial resistance has encouraged the poultry industry to explore alternative strategies that can control pathogenic infections while maintaining immune competence. One promising approach is the utilization of Immunoglobulin Y (IgY), a specific antibody naturally deposited in egg yolks of hens immunized with particular antigens. IgY possesses several advantages, including high antigen specificity, safety, and minimal cross-reactivity with mammalian immune systems, making it a potential candidate for passive immunotherapy in poultry [4].



Previous investigations have demonstrated the antibacterial potential of IgY against various enteric pathogens^[5,6] such as *Escherichia coli*, *Salmonella* spp., and *Clostridium perfringens*. These studies indicate that IgY can provide protective effects against intestinal infections. Nevertheless, most research has focused mainly on its antimicrobial activity *in vitro* or within the intestinal lumen. Limited information is available regarding its systemic immunological effects, particularly its influence on primary lymphoid organs such as the Bursa of Fabricius.

A major limitation of orally administered IgY is its susceptibility to degradation by gastric enzymes, which may reduce its biological activity. To address this issue, encapsulation technologies have been developed to protect IgY during passage through the gastrointestinal tract and enhance its stability and bioavailability^[7]. Chitosan, a natural polysaccharide derived from chitin, has received considerable attention as a drug delivery material due to its biocompatibility, biodegradability, low toxicity, and chemical stability^[8,9]. Furthermore, the presence of reactive functional groups allows chitosan to be modified into various drug delivery systems such as nanoparticles, microparticles, and hydrogels capable of protecting bioactive compounds from harsh gastrointestinal conditions^[9].

Despite advances in encapsulation technology, information regarding the effects of encapsulated IgY on both serological immune responses and histopathological changes in the Bursa of Fabricius remains limited, particularly in broiler chickens challenged with *S. Pullorum*. Since antibody titers reflect the activity of the humoral immune system, which is closely related to the integrity of the Bursa of Fabricius, understanding this relationship is essential.

Therefore, this study aimed to evaluate the influence of orally administered encapsulated IgY on antibody titers and the histological condition of the Bursa of Fabricius in broiler chickens experimentally infected with *S. Pullorum*. Through combined serological and histopathological analyses, this research seeks to clarify the role of IgY in strengthening humoral immunity while preserving lymphoid tissue architecture.

MATERIAL AND METHODS

Ethical Statement

This study obtained ethical clearance from the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Airlangga University (Approval No. 1.KEH.099.07.2024). All experimental procedures were carried out in compliance with established guidelines for the ethical use and welfare of experimental animals.

Animal Preparation

Day-old Cobb 500 broiler chicks (Cobb-Vantress) were maintained in a closed-house system and acclimatized for seven days. During the adaptation period, birds were provided with uniform feed and water. Feed was administered at a controlled amount of 23 g per bird per day, while drinking water was supplied *ad libitum*. Environmental conditions, particularly brooder temperature, were monitored hourly and adjusted according to chick behavior to ensure optimal comfort.

IgY Extraction and Purification

IgY extraction was performed using hyperimmune egg yolks specific to *Salmonella Pullorum*. Egg yolks were separated from albumen and diluted with distilled water at a ratio of 1:8 (v/v). The solution was adjusted to pH 5.0 and subsequently stored in a freezer for 24 h. After thawing, the mixture was centrifuged at 13,500 rpm for 15 min at 4°C to obtain the water-soluble fraction (WSF). The WSF was then adjusted with NaCl solution to pH 4.0 and centrifuged again to obtain the precipitate, which was subsequently dissolved in phosphate-buffered saline (PBS) and adjusted to pH 7.0^[10].

IgY Encapsulation

Encapsulation was performed using calcium chloride (CaCl₂), chitosan, sodium alginate, and purified IgY/yolk preparation at a volume ratio of 1:1:1:1. For each 100 mL formulation, 3.2% CaCl₂ (w/v; approximately 0.29 M), 2% chitosan (w/v), 0.1% sodium alginate (w/v), and 2% purified IgY (w/v) were used. Purified IgY was gradually added to the alginate-CaCl₂-chitosan mixture using a micropipette and homogenized with a magnetic stirrer. The resulting suspension was filtered, rinsed with distilled water, and further homogenized for 15-20 min to obtain encapsulated IgY particles^[10].

Propagation of *Salmonella Pullorum* Colonies

The *S. Pullorum* isolate (strain QJ-2D-Sal) was subcultured on *Salmonella*-Shigella Agar (SSA) and incubated for 24 h^[11]. Colonies were harvested using a sterile inoculating loop and suspended in PBS. The suspension turbidity was adjusted to match McFarland standard I (3×10⁸ CFU) and subsequently diluted to achieve a final concentration of 1.5×10⁸ CFU^[12].

Treatment of Experimental Animals

Twenty-five broiler chickens aged seven days were randomly allocated into five groups. The negative control group (K-) received neither IgY nor infection, while the positive control group (K+) was infected with *Salmonella Pullorum* without IgY treatment. The remaining groups (P1, P2, and P3) received encapsulated IgY based on the average body weight of the chickens on day 7, which was

162 grams with an effective IgY dosage of 700 mg/kg^[13]. The administered doses were 700 mg/kg BW for P1, 1400 mg/kg BW for P2, and 2100 mg/kg BW for P3. These body-weight-based doses were converted using a stock solution (1000 mg/mL) into absolute volumetric doses of 0.11 mL, 0.22 mL, and 0.34 mL per bird per day, respectively, followed by bacterial infection. IgY administration was performed orally each morning after feeding. Infection with *Salmonella Pullorum* was performed at 22 days of age via intramuscular injection into the *musculus pectoralis major* using a bacterial dose of 1.5×10^8 CFU^[14,15].

Organ Sample Collection

Sampling was performed when the chickens reached 25 days of age. Birds were euthanized by cervical dislocation, and the Bursa of Fabricius was collected through dissection and preserved in 10% formalin solution for histological examination. Blood samples were obtained from the brachial vein using sterile syringes and stored in EDTA tubes.

Agar Gel Precipitation Test (AGPT)

The AGPT method was used to detect the presence of specific antibodies in serum samples. A 1% agarose gel prepared in PBS was poured into Petri dishes and allowed to solidify. Wells with a diameter of 3 mm were created in a radial arrangement. Antigen was placed in the central well, while serum samples were added to the surrounding wells. Plates were incubated in a humid chamber at room temperature for 24-48 h. The appearance of a precipitation line between antigen and antibody wells indicated a positive reaction^[16].

Histopathological Observation

Histological sections of the Bursa of Fabricius were examined under a light microscope at 100× magnification across five different fields. Observed parameters included follicular cell depletion, lymphoid cell necrosis, and plica diameter. Histopathological scoring followed the method described by Lukert and Saif. Follicular depletion and necrosis were categorized into four levels: score 0 (no lesion), score 1 (mild; 1-30%), score 2 (moderate; 31-50%), and score 3 (severe; ≥51%).

Statistical Analysis

Non-parametric methods were employed as histopathological scores represent ordinal data and do not follow a normal distribution. Specifically, histopathological score data were analyzed using the Kruskal-Wallis test. When significant differences ($P < 0.05$) were detected, pairwise comparisons were conducted using the Mann-Whitney test. Quantitative statistical analyses were performed using SPSS, version 29.0 (IBM Corp), while qualitative observations were interpreted descriptively.

RESULTS

Agar Gel Precipitation Test (AGPT)

The AGPT evaluation was conducted at 7, 14, 21 and 28 days following the administration of encapsulated yolk immunoglobulin (IgY) (Fig. 1). As presented in Table 1, the extracted IgY demonstrated the capability to generate a specific antibody response against *Salmonella Pullorum*, with antibody titers reaching $2^4 \times 10^5$.

Depletion Follicular

The analysis of follicular depletion revealed significant variation among the experimental groups (K-, K+, P1, P2, and P3) in the Bursa of Fabricius (Fig. 2). As shown in Table 2 statistical analysis indicated no significant differences ($P < 0.05$) in follicular depletion between several group comparisons, including K- (1.80±0.14) with P1 (2.00±0.37), K- with P2 (1.80±0.20), K- with P3 (1.56±0.26), P1 with P2, P1 with P3, and P2 with P3. In contrast, significant differences ($P > 0.05$) were identified between the negative control group (K-; 1.80±0.14) and the positive control group (K+; 2.80±0.14), as well as between the positive control (K+) and all treatment groups, including P1 (2.00±0.37), P2 (1.80±0.20), and P3 (1.56±0.26).

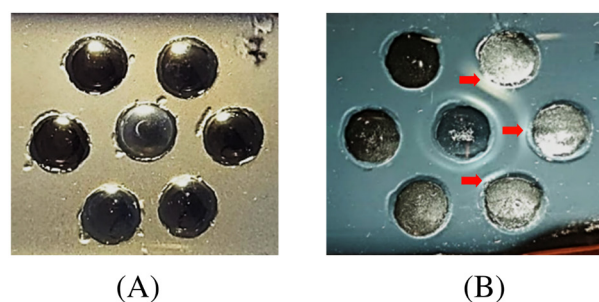


Fig 1. AGPT Results (A) IgY Extraction and (B) IgY Encapsulated IgY Post 4 weeks. The red arrow indicates the presence of precipitation signs near the AGPT test well

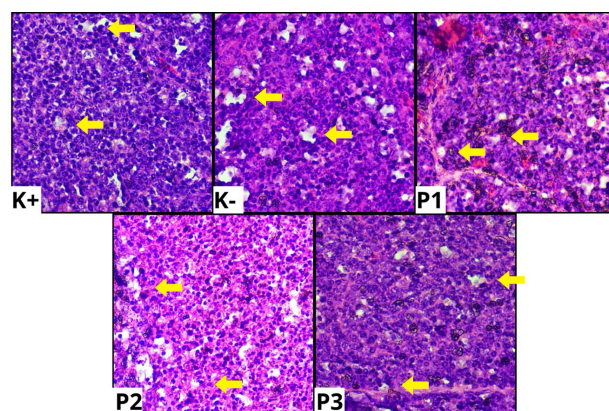


Fig 2. Depletion of Bursa Fabricius. The yellow arrow is depletion of Bursa Fabricius

Table 1. The results titer agar gel precipitation test

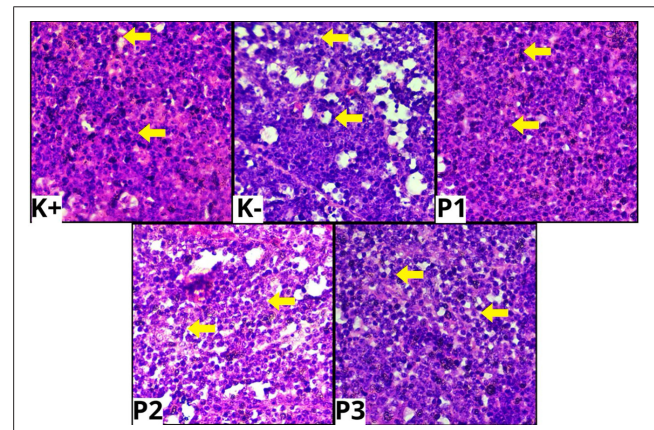
Time	Well	Dilution	Precipitation Line	Titer
24 hours	A1	1:1	No	-
	A2	1:2	No	-
	A3	1:4	No	-
	A4	1:8	No	-
	A5	1:16	No	-
	A6	1:32	No	-
1 week	B1	1:1	Yes	-
	B2	1:2	Yes	-
	B3	1:4	Yes	-
	B4	1:8	No	-
	B5	1:16	No	-
	B6	1:32	No	-
2 weeks	C1	1:1	Yes	-
	C2	1:2	Yes	-
	C3	1:4	Yes	-
	C4	1:8	No	-
	C5	1:16	No	-
	C6	1:32	No	-
3 weeks	D1	1:1	Yes	-
	D2	1:2	Yes	-
	D3	1:4	Yes	-
	D4	1:8	No	-
	D5	1:16	No	-
	D6	1:32	No	-
4 weeks	E1	1:1	Yes	200.000
	E2	1:2	Yes	400.000
	E3	1:4	Yes	1.600.000
	E4	1:8	No	-
	E5	1:16	No	-
	E6	1:32	No	-

Necrosis

Analysis of the necrosis parameter yielded a P-value of <0.05 , indicating the presence of significant differences among the experimental groups (K-, K+, P1, P2, and P3) in terms of lymphoid cell necrosis (Fig. 3). To determine which groups differed significantly, a Mann-Whitney post hoc test was performed. The results showed that there were no statistically significant differences ($P>0.05$) between the K- group (1.72 ± 0.27) and either the P1 (1.92 ± 0.11) or P2 (1.64 ± 0.17) groups. However, significant differences ($P>0.05$) were detected between K- (1.72 ± 0.27) and K+ (2.60 ± 0.20), as well as between K- and P3 (1.36 ± 0.17). Additional significant differences ($P>0.05$) were also identified between the positive control group (K+; 2.60 ± 0.20) and all treatment groups: P1 (1.92 ± 0.11), P2 (1.64 ± 0.17), and P3 (1.36 ± 0.17). Furthermore, comparisons among the treatment groups themselves (P1 vs P2, P1 vs P3, and P2 vs P3) also demonstrated statistically significant differences among the groups ($P>0.05$).

Plica Diameter

The analysis of the plica diameter parameter produced a P-value of >0.05 , indicating significant differences among the experimental groups (K-, K+, P1, P2, and P3) with respect to the plica diameter of the Bursa of Fabricius (Fig.

**Fig 3.** Necrosis of Bursa Fabricius. The yellow arrow is necrosis cell of Bursa Fabricius**Table 2.** Mean scores of follicular depletion, necrosis, and plica diameter in the bursa of Fabricius of the control and treatment groups

Group	Mean Depletion Value $\pm$ SD	Mean Necrosis Value $\pm$ SD	Mean Plica Diameter Value $\pm$ SD (μ m)
Negative Control (K-)	1.80 ± 0.14^a	1.72 ± 0.27^{ac}	232.85 ± 6.59^{ac}
Positive Control (K+)	2.80 ± 0.14^b	2.60 ± 0.20^b	297.71 ± 20.70^b
Treatment 1 (P1)	2.00 ± 0.37^a	1.92 ± 0.11^c	243.02 ± 9.50^c
Treatment 2 (P2)	1.80 ± 0.20^a	1.64 ± 0.17^a	224.78 ± 20.07^a
Treatment 3 (P3)	1.56 ± 0.26^a	1.36 ± 0.17^d	161.59 ± 35.24^d

Data are presented as mean $\pm$ SD. K-, negative control; K+, positive control; P1, P2, and P3, treatment groups receiving 0.11, 0.22, and 0.34 mL/day of encapsulated IgY, respectively. Different superscript letters (a, b, c, d) within a column indicate significant differences based on the Mann-Whitney test ($P<0.05$)

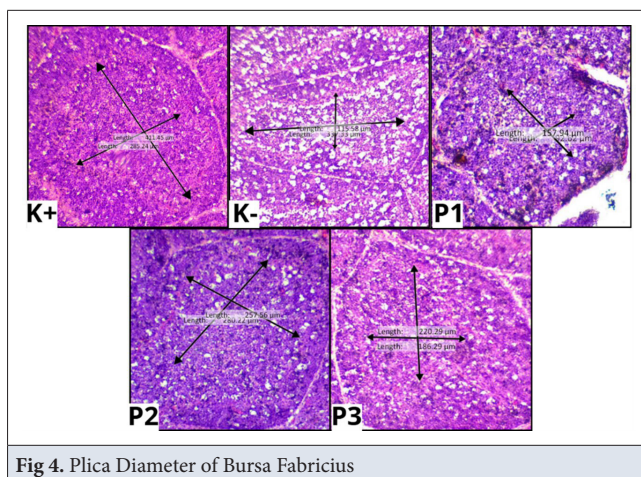


Fig 4. Plica Diameter of Bursa Fabricius

4). Further pairwise comparisons revealed that there were no significant differences ($P < 0.05$) between the K- group ($232.85 \pm 6.59 \mu\text{m}$) and the P1 group ($243.02 \pm 9.50 \mu\text{m}$), nor between K- and P2 ($224.78 \pm 20.07 \mu\text{m}$). In contrast, significant differences ($P > 0.05$) were observed between K- ($232.85 \pm 6.59 \mu\text{m}$) and K+ ($297.71 \pm 20.70 \mu\text{m}$), as well as between K- and P3 ($161.59 \pm 35.24 \mu\text{m}$). Significant differences were also found between the positive control group (K+; $297.71 \pm 20.70 \mu\text{m}$) and each treatment group P1 ($243.02 \pm 9.50 \mu\text{m}$), P2 ($224.78 \pm 20.07 \mu\text{m}$), and P3 ($161.59 \pm 35.24 \mu\text{m}$). Moreover, comparisons among the treatment groups themselves (P1 vs P2, P1 vs P3, and P2 vs P3) also showed statistically significant differences ($P > 0.05$).

DISCUSSION

As shown in Fig. 1, administration of encapsulated IgY 24 hours after treatment did not produce a detectable increase in antibody titers in the blood of broiler chickens. A similar pattern was observed on days 7, 14, and 21, with no precipitation lines detected on the AGPT agar medium, indicating that a measurable specific immune response against *S. Pullorum* had not yet developed. On day 28 (Table 1), an increase in the specific antibody titer against *S. Pullorum* was observed. These findings correspond with the report of Schat et al.^[17], who stated that antibody production following infection with *S. Pullorum* generally becomes detectable only after approximately three weeks. This condition may be associated with the biological nature of encapsulated IgY, which acts as a passive antibody. Such antibodies become functionally active primarily when the pathogen is present in the host system. In contrast, active immunization typically requires repeated booster administration to stimulate the host immune system and induce endogenous antibody production that can be detected serologically. Unlike conventional vaccines, which introduce antigens to stimulate an active immune response, orally administered encapsulated IgY acts

within the gastrointestinal lumen through mechanisms such as inhibition of microbial adhesion, bacterial agglutination or immobilization, and neutralization of toxins and virulence factors, thereby reducing pathogen colonization and burden within the gastrointestinal tract. According to Rahman et al.^[18], orally administered IgY is not translocated across the intestinal epithelium into the bloodstream in its intact molecular form; therefore, the primary effects of oral IgY are considered to be localized within the gastrointestinal tract. Accordingly, in the present study, the protective effect against *Salmonella Pullorum* infection can primarily be explained by the local interaction of IgY with the pathogen within the gastrointestinal tract.

The administration of encapsulated IgY demonstrated a protective effect by reducing follicular cell depletion in broiler chickens challenged with *S. Pullorum*. In the P1 treatment group, which received encapsulated IgY at a dose of 0.11 mL per chicken together with bacterial infection, the mean depletion score was 2.00 ± 0.37 . In comparison, the positive control group (K+), which was infected with *Salmonella Pullorum* without IgY treatment, exhibited a higher depletion score of 2.80 ± 0.14 . These observations are consistent with the findings reported by Wu et al.^[19], indicating that infection with *Salmonella Pullorum* can progressively disrupt lymphoid tissue architecture. The administration of IgY appears to mitigate such damage in infected chickens. This protective effect occurs because IgY functions as a passive antibody that becomes active when pathogenic microorganisms invade the host, thereby helping to limit the extent of lymphoid tissue injury.

The lowest mean necrosis score was observed in the P3 treatment group, which received encapsulated IgY at a dose of 0.34 mL per chicken in combination with *S. Pullorum* infection (1.36 ± 0.17) (Table 2). These results are supported by the work of Carlander et al.^[20], who reported that IgY administration can downregulate the expression of inflammatory-related genes and contribute to the stabilization of systemic immune responses. Encapsulated IgY may suppress excessive inflammatory reactions within the host, thereby preventing the progression of infection and limiting tissue damage. In addition, the ability of IgY to stabilize systemic immune responses may also contribute to improved tissue repair and accelerated recovery processes in infected chickens.

In the P1 and P2 treatment group (Table 2), which received dose of encapsulated IgY (0.11 mL and 0.22 mL per chicken) along with *S. Pullorum* infection, the plica diameter was recorded, with an average value of $243.02 \pm 9.5 \mu\text{m}$ (P1) and $224.02 \pm 20 \mu\text{m}$ (P2). Statistical analysis indicated that this group differed significantly ($P > 0.05$) from the negative control group (K-). The improvement in plica diameter suggests structural recovery of the lymphoid tissue mediated

by the protective activity of IgY antibodies. Liu et al.^[21] also reported that effective suppression of inflammation can facilitate tissue restoration and return the organ to its normal physiological state. Furthermore, a positive association was observed between increasing doses of encapsulated IgY and improved immunological outcomes, including reduced inflammation and regeneration of the plica structure in the Bursa of Fabricius. Encapsulated IgY works by providing natural passive antibodies that strengthen host defense mechanisms against pathogenic invasion. The encapsulation process further enhances this effect by protecting IgY during passage through the digestive tract and enabling targeted release at the appropriate site, thereby improving its ability to neutralize pathogens and reduce tissue injury^[22]. Increasing doses of encapsulated IgY were positively correlated with greater immunological benefits, including reduced lymphoid cell necrosis and enhanced regeneration of lymphoid tissues within the Bursa of Fabricius of broiler chickens infected with *S. Pullorum*. Even the lowest IgY dose (0.11 mL) administered in the P1 group was able to provide a protective effect by decreasing necrotic lesions, although the effect was less pronounced compared with higher doses. These findings support the presence of a dose-dependent relationship between IgY concentration and its immunoprotective capacity^[23]. Nevertheless, the relationship between dose and therapeutic efficacy is not always linear, as increasing the dose will enhance the therapeutic response only up to a certain optimal level.

Administration of encapsulated IgY in broiler chickens challenged with *Salmonella* Pullorum resulted in improved immunological responses and reduced histopathological damage in the Bursa of Fabricius compared with untreated infected chickens. The effective protective response was observed at a dose of 0.11 mL and 0.22 mL per chicken per day, indicating a dose-dependent immunoprotective effect of encapsulated IgY.

Several limitations should be considered when interpreting the findings of the present study. First, the relatively small sample size ($n = 5$ per group) may limit the statistical power and generalizability of the findings. Second, the relatively short observation period, with infection performed at 22 days of age and organ sampling conducted at 25 days of age, represents an additional limitation. A 3-day post-infection period may capture the early pathological response but may not be sufficient to fully characterize the progression of bursal atrophy or subsequent tissue regeneration. Therefore, the findings should be interpreted within the context of the experimental timeline. Future studies involving larger sample sizes and longer post-infection observation periods, including multiple sampling time points, are warranted to better characterize

the progression of bursal lesions and the subsequent tissue recovery following infection.

DECLARATIONS

Availability of Data and Materials: The datasets generated and analysed during the current study are available from the corresponding author (LM) on reasonable request res.

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Competing Interest: The authors declared that there is no conflict of interest.

Declaration of Generative Artificial Intelligence (AI): The authors declare that the article, tables and figures were not written/created by AI and AI-assisted Technologies.

Ethical Compliance Declaration: This study obtained ethical clearance from the Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Airlangga University (Approval No. 1.KEH.099.07.2024). All experimental procedures were carried out in compliance with established guidelines for the ethical use and welfare of experimental animals

Author Contributions: All authors contributed to the study conception and design. RP, VFNR, NHLT, and AIZ: Conceptualization, Investigation, Methodology, Formal analysis, Writing - original draft. LM: Conceptualization, Supervision, Validation, Writing - original draft, Writing - review & editing. EKS: Supervision, Validation, Writing - review & editing. DS, NDK, and AJ: Resources, Methodology, Validation.

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