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Immunomodulatory Effects of IL-4–Expressing Eimeria mitis on Host Hematology and Systemic Immune Parameters in Chickens

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Abstract

Genetically modified *Eimeria* species expressing host cytokines represent a promising strategy for developing live vaccines with intrinsic immunomodulatory properties. In this study, the effects of infection with an interleukin-4 (IL-4)–expressing transgenic *Eimeria mitis* strain on host hematological and systemic immune parameters were evaluated and compared with those induced by wild-type *E. mitis*. Chickens were experimentally infected with either the transgenic or wild-type parasite, and blood samples were collected at defined time points post-infection to assess changes in red and white blood cell indices, hemoglobin concentration, packed cell volume, and leukocyte profiles. Infection with the IL-4–expressing *E. mitis* resulted in distinct hematological patterns compared with wild-type infection. Birds infected with the transgenic strain exhibited less pronounced reductions in red blood cell count, hemoglobin concentration, and packed cell volume, indicating reduced physiological stress and milder systemic impact. Additionally, alterations in total leukocyte counts and differential leukocyte populations suggested a modulation of immune responses associated with IL-4 expression. The observed hematological responses indicate that expression of IL-4 by transgenic *E. mitis* influences host systemic physiology beyond the intestinal site of infection. The combination of reduced parasite fecundity, attenuated pathology, and moderated hematological disturbance supports the concept that IL-4–expressing *E. mitis* functions as a biologically safer live parasite. These findings provide important evidence that cytokine-

expressing *Eimeria* strains can modulate host immune and physiological responses, reinforcing their potential application as next-generation live vaccine vectors in poultry.

1. Introduction

Coccidial infections caused by *Eimeria* species primarily affect the intestinal tract of chickens; however, their impact extends beyond local tissue damage and can induce systemic physiological and immunological changes [1]. Alterations in hematological parameters, including red and white blood cell indices, hemoglobin concentration, and leukocyte distribution, are commonly associated with coccidiosis and reflect the host's response to infection, inflammation, and tissue injury. Monitoring these blood-based indicators provides valuable insight into disease severity, immune activation, and overall host health [2].

Among the *Eimeria* species infecting poultry, *Eimeria mitis* is considered relatively low in pathogenicity, yet infection can still provoke measurable physiological stress and immune responses [3]. Even subclinical infections may disrupt normal hematological balance through intestinal epithelial damage, nutrient malabsorption, and immune-mediated processes. Consequently, hematological analysis is frequently used as a sensitive tool to evaluate the systemic effects of coccidial infections and to assess the safety of live vaccine candidates [4, 5].

Advances in genetic engineering have enabled the development of transgenic *Eimeria* strains capable of expressing host-derived cytokines with the aim of modulating immune responses during infection. Interleukin-4 (IL-4) is a multifunctional cytokine that plays a central role in regulating immune homeostasis [6]. It promotes B-cell differentiation, enhances antibody production, and influences leukocyte recruitment and activation. Importantly, IL-4 is also involved in moderating inflammatory responses, which can help limit tissue damage while preserving protective immunity [7].

Previous studies using exogenous cytokine delivery or plasmid-based expression have demonstrated that IL-4 can influence immune parameters and improve host responses during coccidial infection. However, systemic delivery methods are often limited by short cytokine half-life and uneven tissue distribution. Expression of IL-4 directly by a live *Eimeria* parasite offers a targeted and sustained method of cytokine delivery within the intestinal environment, potentially influencing both local and systemic immune responses [8].

While the immunological benefits of IL-4 expression are increasingly recognized, less attention has been given to its effects on host hematology during infection with transgenic *Eimeria*. Hematological disturbances such as anemia, leukocytosis, and shifts in differential leukocyte populations are commonly associated with coccidiosis and can serve as indicators of disease burden and inflammatory stress. Evaluating these parameters is therefore essential when assessing the biological safety and immunomodulatory impact of genetically modified parasite strains [9].

The present study was designed to investigate how infection with an IL-4–expressing transgenic *E. mitis* strain influences hematological and systemic immune parameters in chickens compared with infection by the wild-type parasite. By analyzing red blood cell indices, hemoglobin levels, packed cell volume, and leukocyte profiles, this work aims to determine whether IL-4 expression mitigates systemic physiological disruption and promotes a more regulated immune response. Understanding these effects is critical for validating IL-4–expressing *E. mitis* as a safe and effective live vaccine vector.

2. Materials and Methods

2.1 Parasite Strains and Preparation of Inocula

Two strains of *Eimeria mitis* were used throughout the study: a wild-type strain and a genetically modified strain expressing chicken interleukin-4 (IL-4). Both strains were maintained under

identical laboratory conditions through serial passage in coccidia-free chickens. Oocysts were recovered from feces, purified by flotation, washed repeatedly in distilled water, and sporulated in potassium dichromate solution under controlled temperature and aeration. Prior to infection, sporulated oocysts were washed thoroughly to remove residual dichromate and quantified using a hemocytometer. Inocula were prepared freshly on the day of infection to ensure parasite viability. Equal numbers of viable sporulated oocysts were administered to each bird in the infected groups to allow direct comparison between wild-type and IL-4–expressing parasites.

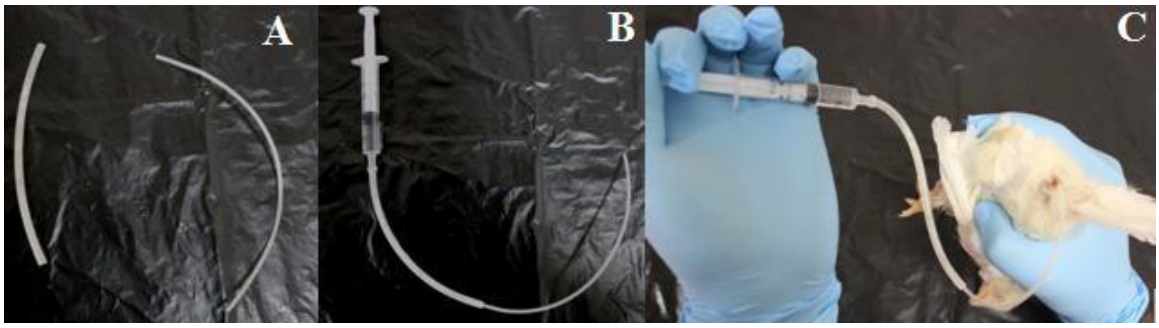

Figure 6: Set up for predilection site inoculation of *E. mitis*:
A. Different sizes of clear silicone rubber tubing, B. Inoculation tool assembly, C. Chicken inoculation

2.2 Experimental Birds and Housing Conditions

One-day-old Arbor Acres broiler chickens were obtained from a commercial hatchery and reared under strict hygienic conditions. Birds were housed in isolators with controlled temperature, humidity, and lighting. Feed and water were provided ad libitum, and birds were monitored daily to ensure normal growth and health status. Before initiation of the experiment, birds were confirmed to be free of coccidial infection through fecal examination. At the designated age, chickens were randomly allocated into three experimental groups: an uninfected control group, a wild-type *E. mitis*–infected group, and an IL-4–expressing *E. mitis*–infected group. Each group was housed separately to prevent cross-infection.

2.3 Infection Protocol

Chickens in the infected groups were orally inoculated with a defined dose of sporulated oocysts using a sterile gavage needle. The infection dose was selected based on preliminary trials showing consistent establishment of infection without inducing severe clinical disease or mortality. Control birds received an equivalent volume of sterile phosphate-buffered saline. Following infection, birds were observed daily for clinical signs, including changes in behavior, feed intake, and fecal consistency. No anticoccidial drugs or immunomodulatory treatments were administered during the experimental period.

2.4 Blood Sample Collection

Blood samples were collected from the wing vein of randomly selected birds from each group at predetermined time points corresponding to the active phase of parasite development and oocyst shedding. Sampling times were chosen to capture hematological changes associated with both early and peak stages of infection. Blood was collected into tubes containing anticoagulant to prevent clotting. Samples were processed immediately after collection to ensure accurate measurement of cellular components.

2.5 Determination of Red Blood Cell Parameters

Red blood cell–related indices were measured using standard veterinary hematological techniques. These included:

- Red blood cell (RBC) count, determined using a hemocytometer after appropriate dilution
- Hemoglobin concentration (Hb), measured using colorimetric methods
- Packed cell volume (PCV), determined by microhematocrit centrifugation

These parameters were used to evaluate anemia, blood loss, and general physiological stress associated with *E. mitis* infection.

2.6 Determination of White Blood Cell Parameters

Total white blood cell (WBC) counts were determined using diluted blood samples and hemocytometer-based counting methods. To assess differential leukocyte profiles, thin blood smears were prepared immediately after sampling, air-dried, fixed, and stained using standard hematological stains. Differential counts were performed under light microscopy by examining multiple microscopic fields per smear. Leukocytes were classified as lymphocytes, heterophils, monocytes, eosinophils, or basophils, and their relative proportions were calculated. These values were used to assess immune activation and systemic inflammatory responses.

2.7 Assessment of Systemic Immune Modulation

Changes in hematological parameters were interpreted as indicators of systemic immune and physiological responses to infection. Comparisons were made between the wild-type and IL-4–expressing *E. mitis* groups to determine whether IL-4 expression influenced the magnitude or nature of hematological alterations.

Particular attention was given to:

- preservation or reduction of RBC indices
- magnitude of leukocyte responses
- shifts in lymphocyte and heterophil populations

These patterns were used to infer immunomodulatory effects associated with IL-4 expression during infection.

2.8 Data Analysis

Hematological data were summarized as mean values for each experimental group. Trends in red and white blood cell parameters were compared among groups to identify differences attributable to parasite strain. Observations were interpreted in relation to infection status, parasite fecundity, and previously observed biological characteristics of the transgenic line.

3. Results

3.1 General Clinical Observations

Chickens infected with either wild-type or IL-4–expressing *Eimeria mitis* remained active throughout the experimental period, and no mortality was observed. Mild, transient changes in fecal consistency were noted in infected birds during peak parasite development, but these signs were less pronounced in the group infected with the IL-4–expressing strain. Uninfected control birds showed no clinical abnormalities.

Table 3.1. General clinical observations of chickens following infection with wild-type and IL-4–expressing *Eimeria mitis*

Experimental group	Activity level	Mortality	Fecal consistency changes	Overall clinical signs
Uninfected control	Normal, active	None (0%)	None observed	No clinical abnormalities
Wild-type *E. mitis* infected	Active	None (0%)	Mild, transient changes during peak parasite development	Mild clinical signs
IL-4–expressing *E. mitis* infected	Active	None (0%)	Very mild and transient changes; less pronounced than wild-type group	Minimal clinical signs

3.2 Red Blood Cell Indices

3.2.1 Red Blood Cell Count

Infection with wild-type *E. mitis* resulted in a noticeable reduction in red blood cell (RBC) counts during the active phase of infection. This decline was most evident around the peak period of parasite development and oocyst shedding. In contrast, chickens infected with the IL-4–expressing *E. mitis* exhibited a smaller decrease in RBC counts. Although values in the transgenic group were lower than those of uninfected controls, they remained consistently higher than those observed in the wild-type group. These findings indicate that infection with the IL-4–expressing parasite caused less disruption to erythrocyte homeostasis, suggesting reduced physiological stress and milder systemic effects.

3.2.2 Hemoglobin Concentration

Hemoglobin (Hb) concentration followed a pattern similar to that of RBC counts. Birds infected with wild-type *E. mitis* experienced a measurable reduction in Hb levels during the peak infection period. In contrast, the IL-4–expressing group showed a more moderate decline, with Hb concentrations remaining closer to baseline values. The preservation of hemoglobin levels in the IL-4 group suggests that cytokine expression may mitigate infection-associated anemia, potentially by reducing intestinal damage, blood loss, or inflammatory-mediated suppression of erythropoiesis.

3.2.3 Packed Cell Volume

Packed cell volume (PCV) values were significantly affected by wild-type *E. mitis* infection, with clear reductions observed during peak parasitic activity. Chickens infected with the IL-4–expressing parasite exhibited higher PCV values compared with the wild-type group at corresponding time points. Although PCV was still lower than that of uninfected controls, the magnitude of reduction was substantially less severe. Together, the RBC count, hemoglobin concentration, and PCV results demonstrate that IL-4 expression in *E. mitis* alleviated the hematological impact typically associated with coccidial infection.

3.3 Total White Blood Cell Response

Total white blood cell (WBC) counts increased in response to infection in both parasite groups, reflecting activation of the host immune system. The magnitude and pattern of this response, however, differed between groups. Birds infected with wild-type *E. mitis* showed a pronounced

increase in total WBC counts during the peak infection period, indicative of a strong systemic inflammatory response. In contrast, chickens infected with the IL-4–expressing strain exhibited a more moderate elevation in WBC counts. This moderated leukocytosis suggests that IL-4 expression tempered excessive systemic inflammation while still allowing immune activation.

Table 3.2. Hematological indices ±SE in birds exposed to wild and transgenic strains of *E. mitis*

	Groups of experimental broiler birds			
Parameter/Days-P.I	**(A)Control**	**(B)Wild**	**(C)Transgenic**	**P-Value**
Erythrocyte(10^6/µl)				
2	3.24±0.03	3.14±0.01	3.2±0.02	0.0782
4	3.24±0.07	3.13±0.01	3.16±0.01	0.067
6	3.29±0.03	2.62±0.03*	3.09±0.01	0.01
Hemoglobin(g/dl)				
2	13.84±0.01	12.58±0.2	13.37±0.25	0.0717
4	13.01±0.24	10.11±0.40*	13.03±0.25	0.010
6	13.3±0.17	9.42±0.09*	12.74±0.33	0.001
	Packed cell volume (%)			
2	34.9±0.53	30.99±0.55	33.56±0.25	0.7231
4	35.1±0.68	28.41±0.46*	33.29±0.17	0.002
6	35.17±0.30	28.14±0.28*	33.6±0.38	0.001
	Mean corpuscular volume (fl)			
2	126.4±0.37	135.0±0.95	125.4±0.63	0.746
4	127.7±0.75	135.2±0.45*	124.5±0.76	0.0001
6	125.2±0.27	135.5±0.57*	123.1±0.27	0.0001
	Mean corpuscular hemoglobin concentration (%)			
2	35.19±0.28	32.3±0.43	33.4±0.13	0.5922
4	34.9±0.45	28.5±0.29*	32.9±0.19	0.0002
6	34.8±0.29	27.9±0.21*	32.7±0.35	0.0001

Values (mean ±SE) in rows differ significantly ($P< 0.05$). The group A served as control while the birds of groups B and C were inoculated with wild and *tE. mitis* respectively.

3.4 Differential Leukocyte Profiles

3.4.1 Lymphocytes

Lymphocyte proportions increased in both infected groups compared with uninfected controls. However, the increase was more pronounced and sustained in birds infected with the IL-4–expressing parasite. This pattern suggests enhanced lymphocyte involvement, consistent with IL-4–mediated stimulation of adaptive immune responses.

3.4.2 Heterophils

Heterophil proportions were elevated in wild-type–infected birds, particularly during the peak infection period. This increase is characteristic of acute inflammatory responses. In contrast, heterophil levels in the IL-4–expressing group were lower and more stable, indicating a reduced inflammatory burden.

3.4.3 Monocytes, Eosinophils, and Basophils

Minor fluctuations in monocyte, eosinophil, and basophil populations were observed in both infected groups. These changes did not differ markedly between wild-type and transgenic infections and remained within physiological ranges. Their contribution to the overall immune response appeared secondary compared with lymphocyte and heterophil dynamics.

Table 3.3. Total and differential leukocyte indices ±SE in broiler birds exposed to wild and *tE. mitis*

	Groups			
Parameter/Days-P.I	**(A)Control**	**(B)Wild**	**(C)Transgenic**	**P-Value**
		Leukocyte counts (10^3/µl)		
2	12.4±0.13	13.7±0.31	12.3±0.05	0.4200
4	12.4±0.21	15.3±0.03*	13.7±0.20	0.0001
6	12.7±0.05	17.5±0.14*	13.9±0.29	0.0001
Heterophil(10^3/µl)				
2	51.4±0.59	54.6±0.87	53.2±0.86	0.6230
4	52.1±0.68	59.8±0.68	53.9±0.83	0.0001
6	53.3±0.37	63.2±0.56*	54.2±0.61	0.0001
		Lymphocyte (10^3/µl)		
2	39.1±0.38	35.9±0.28	37.2±0.33	0.5781
4	38.1±0.57	33.9±0.29*	36.5±0.12	0.0039
6	38.3±0.22	32.9±0.25*	36.7±0.29	0.0007
Monocyte (10^3/µl)				
2	4.33±0.08	4.81±0.02	4.91±0.01	0.0657
4	4.38±0.03	5.39±0.04*	5.13±0.04	0.0002
6	4.57±0.05	5.54±0.02*	5.29±0.02	0.0001
Eosinophil (10^3/µl)				
2	1.91±0.01	2.01±0.03	1.95±0.00	0.4219
4	1.92±0.01	2.35±0.01*	1.96±0.01	0.0001
6	1.95±0.01	2.41±0.02*	1.99±0.00	0.0001
Basophil (10^3/µl)				
2	3.79±0.04	3.84±0.02	3.84±0.02	0.2552
4	3.93±0.01	4.39±0.03*	3.96±0.02	0.0001
6	3.89±0.02	4.68±0.05*	3.97±0.03	0.0001

Values (mean ±SE) in rows differ significantly ($P< 0.05$). Group A served as control while

the birds of groups B and C were inoculated with wild and *tE. mitis* strains respectively.

3.5 Comparison with Uninfected Controls

Uninfected control birds maintained stable hematological parameters throughout the study period. No significant changes in RBC indices, WBC counts, or differential leukocyte profiles were observed. This stability confirms that the hematological changes recorded in infected groups were attributable to *E. mitis* infection and not to environmental or husbandry factors.

4. Discussion

The present study demonstrates that infection with an IL-4–expressing transgenic *Eimeria mitis* strain results in markedly different hematological and systemic immune responses compared with infection by the wild-type parasite. Although both strains successfully established infection, the magnitude and nature of the host response differed substantially, indicating that IL-4 expression by the parasite plays a significant role in modulating systemic physiology during coccidial infection [9].

One of the most notable findings was the reduced severity of changes in red blood cell–related parameters in chickens infected with the IL-4–expressing strain. Wild-type *E. mitis* infection led to clear reductions in red blood cell count, hemoglobin concentration, and packed cell volume, consistent with infection-associated physiological stress and mild anemia. In contrast, birds infected with the IL-4–expressing parasite exhibited smaller declines in these parameters, suggesting that IL-4 expression mitigated the systemic effects typically associated with coccidiosis. Preservation of erythrocyte indices indicates reduced intestinal damage, decreased blood loss, or improved recovery of erythropoiesis during infection [10, 11].

The moderated red blood cell responses observed in the IL-4 group are particularly important in the context of vaccine safety. Hematological disturbances are often linked to reduced growth performance and impaired health in poultry. A live vaccine candidate that limits such disturbances while still stimulating immunity offers a clear advantage. The data indicate that IL-4–expressing *E. mitis* exerts less physiological stress on the host, reinforcing its suitability as a biologically safer live parasite [12].

Differences in white blood cell responses further support the immunomodulatory role of IL-4 expression. While both parasite strains induced leukocytosis, the response was more pronounced in chickens infected with the wild-type strain. Elevated total leukocyte counts and increased heterophil proportions in these birds reflect a strong inflammatory reaction. In contrast, infection with the IL-4–expressing parasite resulted in a more moderate leukocyte response, suggesting controlled immune activation rather than excessive inflammation. This balanced response is desirable for vaccination strategies, as excessive inflammation can contribute to tissue damage and reduced performance [13, 14].

The differential leukocyte profiles observed between groups provide further insight into the nature of immune modulation. Chickens infected with the IL-4–expressing strain exhibited relatively higher lymphocyte proportions and lower heterophil responses compared with the wild-type group. This pattern suggests a shift toward adaptive immune engagement rather than acute inflammatory dominance. Such a shift is consistent with the known biological functions of IL-4, which promotes lymphocyte differentiation and supports antibody-mediated immunity while limiting overly aggressive inflammatory responses [15].

Importantly, the changes observed in hematological parameters occurred without evidence of adverse clinical outcomes. Birds infected with the IL-4–expressing parasite remained active and showed only mild, transient signs of infection. This observation supports the conclusion that IL-4 expression not only modulates immune responses but also contributes to overall host tolerance of

infection. The combination of reduced parasite fecundity, moderated immune activation, and preserved physiological stability underscores the attenuated nature of the transgenic strain [16]. These findings highlight the value of integrating cytokine expression into transgenic *Eimeria* vaccine platforms. By expressing IL-4 directly within the host intestinal environment, the parasite appears to shape systemic immune responses in a way that minimizes pathological consequences while maintaining immunological stimulation. This localized, sustained cytokine delivery strategy offers clear advantages over conventional approaches that rely on external cytokine administration. In conclusion, the hematological and systemic immune responses observed in this study provide strong evidence that IL-4–expressing *E. mitis* induces a more regulated and less disruptive host response than wild-type infection. These characteristics are highly desirable for live vaccine development and support the continued exploration of cytokine-expressing *Eimeria* strains as next-generation immunization tools in poultry.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

All authors contributed equally.

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References

1. Abebe, E. and G. Gugsa, *A review on poultry coccidiosis.* Abyssinia Journal of Science and Technology, 2018. **3**(1): p. 1-12.
2. Gao, Y., et al., *Advancements in understanding chicken coccidiosis: from Eimeria biology to innovative control strategies.* One Health Advances, 2024. **2**(1): p. 6.
3. Xu, L., et al., *Pathogenic effects of single or mixed infections of Eimeria mitis, Eimeria necatrix, and Eimeria tenella in chickens.* Veterinary Sciences, 2022. **9**(12): p. 657.
4. Lillehoj, H. and K. Lee, *Immune modulation of innate immunity as alternatives-to-antibiotics strategies to mitigate the use of drugs in poultry production*. 2012, Elsevier. p. 1286-1291.
5. Adamu, M., et al., *Hematological, biochemical and histopathological changes caused by coccidiosis in chickens.* Agriculture and Natural Resources, 2013. **47**(2): p. 238-246.
6. Hsieh, M.-K., *Approaches to enhance protection against infectious bursal disease in chickens conferred by DNA-mediated vaccination*. 2005, Purdue University.
7. Zheng, L., et al., *Prevention and control of chicken coccidiosis construction of recombinant Lactococcus lactis expressing chicken IL-4 and IL-2 fusion protein and its immune synergistic effect on chicken coccidia live vaccine.* Poultry Science, 2023. **102**(4): p. 102530.
8. Lee, Y., M. Lu, and H.S. Lillehoj, *Coccidiosis: recent progress in host immunity and alternatives to antibiotic strategies.* Vaccines, 2022. **10**(2): p. 215.
9. Khan, J.M., et al., *Transgenic Eimeria mitis Expressing Chicken IL-4 Mediated Decrease in Pathogenicity Compared to Wild Type Eimeria mitis Strains in Broiler Chickens.* Pakistan Veterinary Journal, 2016. **36**(4).
10. Chapman, H.D., et al., *A selective review of advances in coccidiosis research.* Advances in parasitology, 2013. **83**: p. 93-171.
11. Dalloul, R.A. and H.S. Lillehoj, *Recent advances in immunomodulation and vaccination strategies against coccidiosis.* Avian diseases, 2005. **49**(1): p. 1-8.

12. Arczewska-Włosek, A., et al., *Effects of a dietary multi-strain probiotic and vaccination with a live anticoccidial vaccine on growth performance and haematological, biochemical and redox status indicators of broiler chickens.* Animals, 2022. **12**(24): p. 3489.
13. Cupo, K.L., *Immunological Investigation of Protozoan Parasitic Infections in Poultry*. 2022: North Carolina State University.
14. de Wolf, J., *Immune development in poultry against round-and tapeworm infections*. 2023, Ghent University.
15. Chauhan, R.S., et al., *Immunopathology of parasitic diseases of animals*, in *Essentials of veterinary immunology and immunopathology*. 2024, Springer. p. 483-503.
16. Fatoba, A.J. and M.A. Adeleke, *Transgenic Eimeria parasite: A potential control strategy for chicken coccidiosis.* Acta tropica, 2020. **205**: p. 105417.