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Protective Immunity and Vaccine Potential of IL-4–Expressing *Eimeria mitis* Against Homologous Challenge in Chickens

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Abstract

Live Eimeria-based vaccines are still considered one of the best methods of managing avian coccidiosis, but the safety, pathogenicity, and environmental pollution have restricted their wider use. Modification of Eimeria species to express host cytokines is a new method of enhancing the effectiveness of a vaccine and minimizing biological risk. The protective immunity and vaccine potential of an interleukin-4 (IL-4)-expressing transgenic *Eimeria mitis* (*E. mitis*) strain in chicken were assessed in this research against homologous challenge. The IL-4 expressing *E. mitis* strain was immunized in chickens and later challenged by the wild-type *E. mitis*. Protective efficacy was determined through observing the oocyst shedding, clinical signs, and host performance indicators after the challenge. The transgenic strain immunized birds showed a significant decrease in oocyst production at the time of challenge as compared to non-immunized controls, which showed a positive result that the immunization induced successful protective immunity. Besides, there were milder clinical outcomes and more stable physiological parameters of immunized birds, which is consistent with improved immune response. The IL-4 *E. mitis* strain showed a beneficial ratio between attenuation and immunogenicity. There was less fecundity and slower shedding, which minimized environmental contamination, and the growth of parasites was a guarantee of strong immune stimulation. The findings demonstrate that IL-4 gene expression improves the vaccine capacity of *E. mitis* through protective immunity with reduced host pathology.

1. Introduction

The problem of Avian coccidiosis, which is a protozoan parasite (*Eimeria* genus) remains a significant threat to the poultry industry worldwide. Although chemoprophylaxis and management practices have lowered the severity of the disease, the problem of drug resistance and consumer demand for antibiotic-free production of poultry has led to increased dependence on the use of vaccination methods [1]. Live Eimeria vaccines are the most efficient way to induce long-term immunity; nevertheless, the issue of pathogenicity of the parasite, uncontrolled multiplication, and pollution of the environment restricts the large-scale use [2, 3].

E. mitis is one of the Eimeria species that infect chicken and has a low pathogenicity, allowing it to complete its life cycle and elicit protective immunity. The features presented above render *E. mitis* a good candidate to develop as a live vaccine carrier. Further improvements in genetic manipulation have increased its possibilities by allowing the foreign expression of foreign genes, including host-derived immunomodulatory molecules, into the parasite [4, 5].

Interleukin-4 (IL-4) is a versatile cytokine that is very important in the regulation of the immune response. It stimulates the activities of B-cells, facilitates the formation of antibodies, and is involved in the control of inflammation [6, 7]. IL-4 may be used during intestinal infections to modulate both local and systemic immune environments, increasing the possibility of protective responses and reducing tissue injury. Vaccination of IL-4 with a live Eimeria as a vector provides a specific and sustained route of exposure to cytokines that is not possible by the standard routes of vaccination or aerosol exposure to cytokines [8].

Several studies have established that the cytokine-producing Eimeria strains have distorted biological traits such as decreased fecundity and pathogenicity but retained immunogenicity. All these are very desirable traits in vaccine candidates and help to enhance safety without hindering protective efficacy. The capacity of IL-4 expressing *E. mitis* to cause protective immunity on a new challenge has, however, not been completely described [9, 10].

The measures of protection against coccidiosis are normally determined through an analysis of the decrease in oocyst shedding, relief of clinical symptoms, and enhancement of host physiological stability after challenge infection. Effective vaccines inhibit the replication and spread of parasites and allow the host to develop a fast and regulated response of immune response. Thus, the analysis of these parameters after immunization against transgenic *E. mitis* is a crucial step towards the identification of its practical value as a vaccine [11, 12].

The current research aimed to evaluate the protective immunity of the *E. mitis* strain with a native IL-4 gene against the challenge posed by the homogeneous wild-type. This work will accomplish this by using oocyst output, clinical manifestations, and host responses in immunized and non-immunized chickens to establish whether IL-4 expression increases vaccine effectiveness and avoids safety concerns. The results show valuable evidence on the possibility of developing the avian coccidiosis cytokine-expressing Eimeria as the next-generation of live vaccines.

2. Materials and Methods

2.1 Parasite Strains and Oocyst Preparation

This study involved the use of two *Eimeria mitis* strains, namely a wild-type strain and an interleukin-4 (IL-4)-expressing transgenic strain. Both strains were preserved by serial passage in chickens that were coccidia-free and kept under the same laboratory conditions. The feces were collected at the highest shedding time, purified through flotation, and rinsed with distilled water several times to eliminate fecal debris. The sporulation of purified oocysts in potassium dichromate solution was done at room temperature with continuous aeration until the sporulation process was completed. Before the experiments, sporulated oocysts were washed repeatedly with sterile phosphate-buffered saline to eliminate the presence of dichromate. The concentration of oocysts

was measured with the help of a hemocytometer, and only the oocysts that were fully sporulated and morphologically normal were inoculated.

2.2 Experimental Birds and Housing Conditions

Arbor Acres broiler chickens of one-day-old broilers were sourced at a commercial hatchery and raised in coccidia-free isolators. Conditions that existed in the environment, such as temperature, humidity, and lighting, were put under control during the study. Birds were given a commercial, anticoccidial-free food and water at will. Before the experiment was started, microscopic examination of fecal samples was done to ensure that Eimeria oocysts were absent. Experimental groups were randomly divided, and birds were placed in different isolators to avoid cross-contamination of the treatments.

2.3 Experimental Design and Group Allocation

Chickens were divided into three experimental groups:

1. Uninfected control group, receiving no immunization or challenge
2. Unimmunized–challenged group, receiving wild-type *E. mitis* challenge only
3. IL-4–expressing *E. mitis*–immunized and challenged group

This design allowed assessment of baseline infection dynamics, vaccine-induced protection, and overall safety of the transgenic parasite.

2.4 Immunization Procedure

The birds in the immunized group were administered a sporulated IL-4 expressing *E. mitis* oocysts by oral inoculation with a known dose of oocysts using a sterile gavage needle. The dose of immunization was chosen on the basis of primary research conducted, which had shown consistent infection induction with low clinical effect. Birds were observed on a daily basis after the immunization period on overall health, behaviour, and fecal structure. The immunization phase was marked by the shedding of the oocyst to confirm the replication and attenuation of the parasite. No anticoccidial drugs were given at any time during the experiment.

2.5 Challenge Infection

Following the primary infection and an adequate time in which the birds in the immunized group could be primed, birds in the immunized group were orally challenged with sporulated wild-type *E. mitis* oocysts. The unimmunized challenged group received the same dose at the same point in time in order to make a direct comparison of the birds. Sterile phosphate-buffered saline was given only to control birds. Each inoculation was done in the same condition to reduce variation in the procedure.

2.6 Clinical Monitoring After Challenge

Birds were observed at least twice daily throughout the post-challenge period. Clinical observations included:

- activity level and behavior
- feed intake
- fecal appearance
- presence of diarrhea or abnormal droppings

2.7 Fecal Sample Collection and Processing

The fecal samples were observed on a daily basis per experimental group, starting five days after challenge until the anticipated duration of oocyst shedding. To prevent contamination new

droppings were taken on paper-lined floors of isolators. The homogenization of the samples was performed to achieve an even dispersion of the oocysts. Quantum amount of feces was quantified and suspended in a saturated salt solution of flotation to measure the quantity of oocysts.

2.8 Quantification of Oocyst Shedding

The oocyst shedding was determined by a modified McMaster count method. Counting chambers loaded with homogenized fecal suspensions were counted under light microscopy, and oocysts within the designated grid areas were counted. The number of oocysts per gram of feces was used to express the number of oocysts daily. Daily shedding curves were plotted using these values, and cumulative oocyst output in every experimental group was calculated.

2.9 Assessment of Protective Immunity

Protective efficacy was evaluated by comparing post-challenge oocyst shedding between immunized–challenged and unimmunized–challenged groups. Parameters assessed included:

- onset of oocyst shedding
- peak shedding intensity
- duration of shedding
- cumulative oocyst output

3. Results

3.1 General Health and Survival Following Immunization and Challenge

During the immunization and challenge period of the experiment, all the chickens survived. In any group, there was no mortality. The IL-4 expressing *E. mitis* strain that was used to immunize birds was found to be active and exhibited normal feeding behavior during the primary immunization, in addition to the post challenge period. Softness of feces (mild and temporary) was sometimes observed in the period of optimality of parasites, but it was over without treatment. Conversely, birds that had not received immunization were challenged with wild-type *E. mitis*, which reflected more dramatic clinical characteristics in the post-challenge period, such as decreased activity and loosened feces. The experimental period did not reveal any abnormalities in the uninfected control birds.

Table 1. General health status and survival of chickens following immunization with IL-4–expressing Eimeria mitis and subsequent challenge.

Experimental Group	Immunization Status	Challenge Status	Survival	Clinical Observations During Immunization	Post-Challenge Clinical Signs
IL-4–expressing *E. mitis* immunized birds	Immunized with IL-4–expressing *E. mitis*	Challenged	100% survival (no mortality)	Normal feeding behavior; mild, temporary soft feces during peak parasite activity	No severe clinical signs; fecal softness resolved without treatment
Non-immunized, challenged birds	Not immunized	Challenged with wild-type *E. mitis*	100% survival (no mortality)	Not applicable	Decreased activity and loosened feces observed post-challenge

Uninfected control birds	Not immunized	Not challenged	100% survival (no mortality)	Normal	No abnormalities observed throughout the experimental period

3.2 Oocyst Shedding During the Immunization Phase

Successful immunization of chickens with the IL-4-producing *E. mitis* strain resulted in shedding of oocysts in the primary infection, which validates the successful establishment of the transgenic parasite. Nevertheless, the number of oocysts produced in this stage was significantly less than that which would be expected of wild-type *E. mitis*, which is in line with the weakened phenotype of reproductive fitness of the IL-4-expressing strain. The shedding was in the expected temporal order of *E. mitis*, where oocysts were initially recorded during the period between Days 5 and 6 after immunization and decreased thereafter. The diminished severity of shedding in immunization was a confirmation of both parasite viability and immunization, and was the indicator of immune priming without unnecessary parasite load (Fig 1A &B).

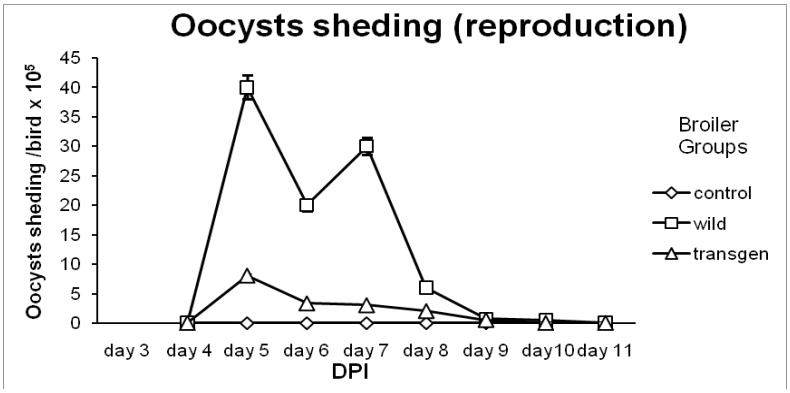
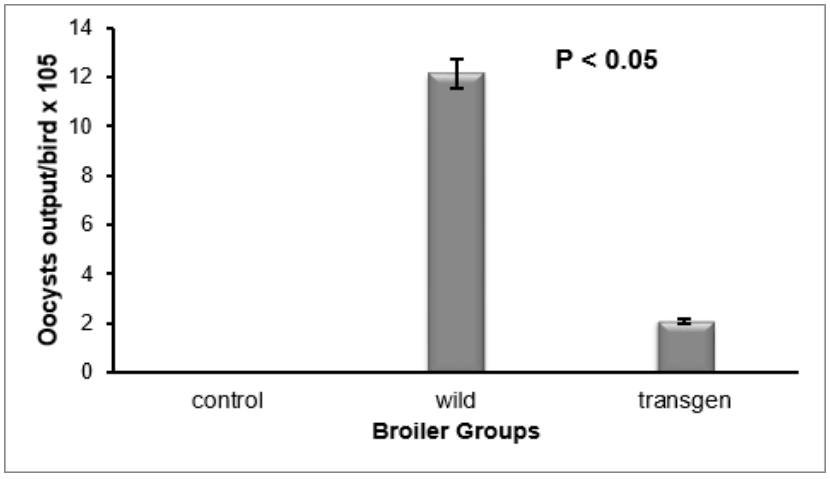


Figure 1A: Comparison of oocytes shedding pattern Experiment 1 Group A, Comparison of oocytes shedding pattern of *tE.mitis* with that of the *wE. mitis*. Oocyst shedding was measured after every 24 hours between day 5 and day 11 post infection. The data represented one of the three independent experiments with almost similar results and are expressed as the mean ± SD.



Mean oocysts output per bird collected from birds treated with EmiChIL-4 and wild type *E. mitis* from 3 to 11 days post infection.

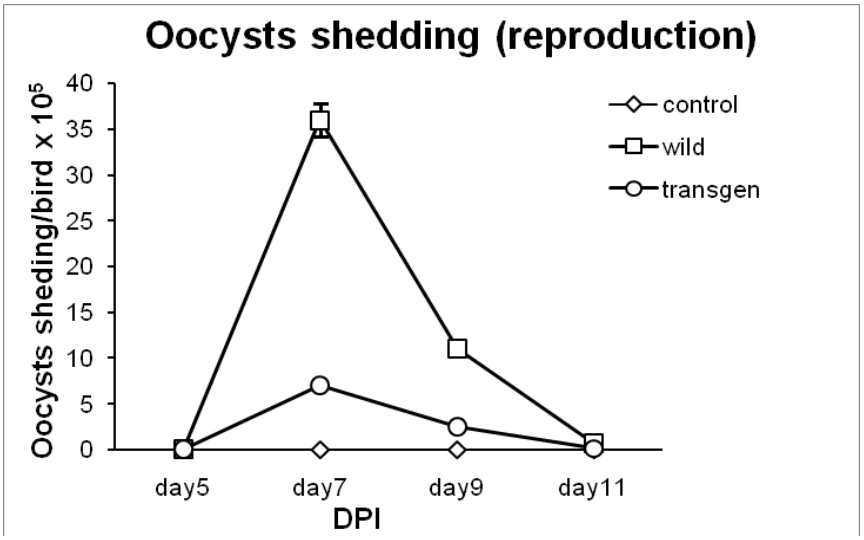
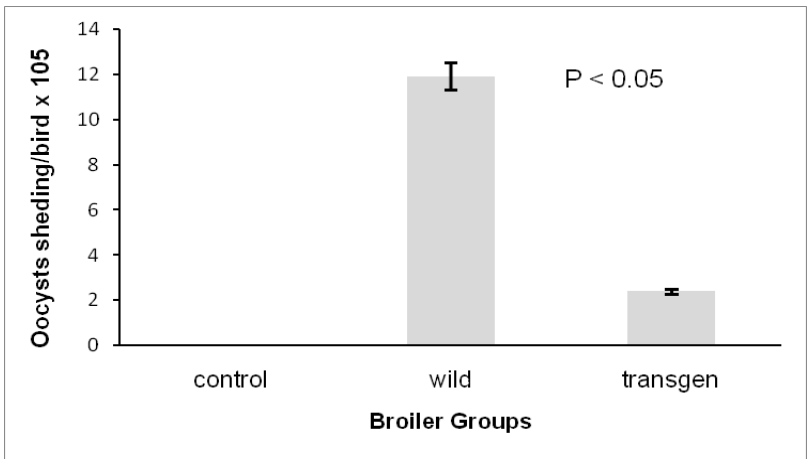


Figure 1B Comparison of oocytes shedding pattern

Second Study Group B, Comparison of oocytes shedding pattern of *tE.mitis* with that of the wild type *E.mitis*. Five birds from the first group were infected with *tE.mitis*. Oocytes shedding were measured on day 5, 7, 9 and day11 post infection. The data represented corresponds to three independent experiments with almost similar results and is expressed as the mean ± SD.



Oocyst output per bird of EmiChIL-4 and *wE. mitis*

Mean total oocyst output per bird of EmiChIL-4 and *wE. mitis* on day 5, 7, 9 and day 11 post infection.

3.3 Oocyst Shedding After Homologous Challenge in Unimmunized Birds

Unimmunized birds challenged with wild-type *E. mitis* displayed a typical infection profile. Oocyst shedding began approximately five days post-challenge and increased rapidly to a clear peak. High daily oocyst counts were recorded over multiple consecutive days, followed by a gradual decline as the infection resolved. The cumulative oocyst output in this group was high, reflecting unrestricted parasite replication and confirming that the challenge dose was sufficient to establish a robust infection in the absence of prior immunity.

3.4 Oocyst Shedding After Homologous Challenge in Immunized Birds

Birds previously immunized with the IL-4–expressing *E. mitis* strain showed a markedly altered response to homologous wild-type challenge. Although oocyst shedding was still detectable, both the intensity and duration of shedding were substantially reduced compared with unimmunized–challenged birds.

In immunized birds:

- The onset of shedding was delayed or occurred at low initial levels
- Peak oocyst shedding was markedly suppressed
- Daily oocyst counts remained consistently low throughout the shedding period
- Shedding declined rapidly after the peak

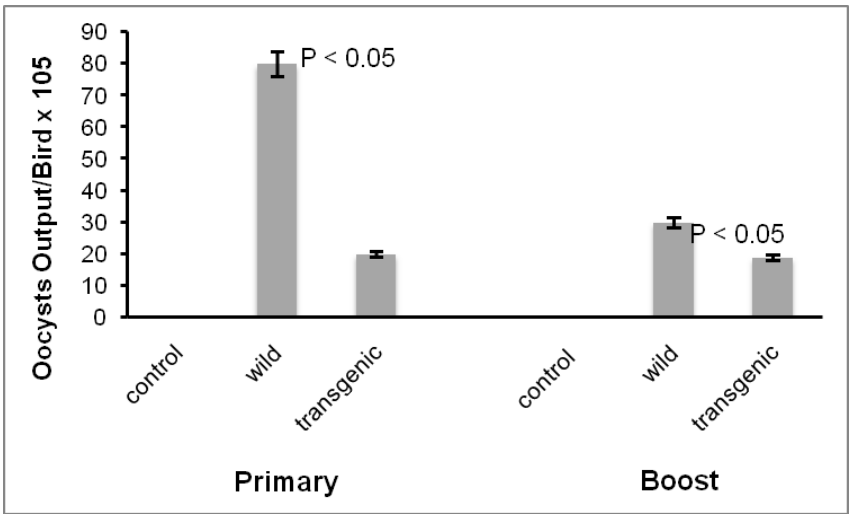


Figure 2A: Oocyst output per bird *(p > 0.05)* after primary and boost immunization with wild type and *tE. mitis*

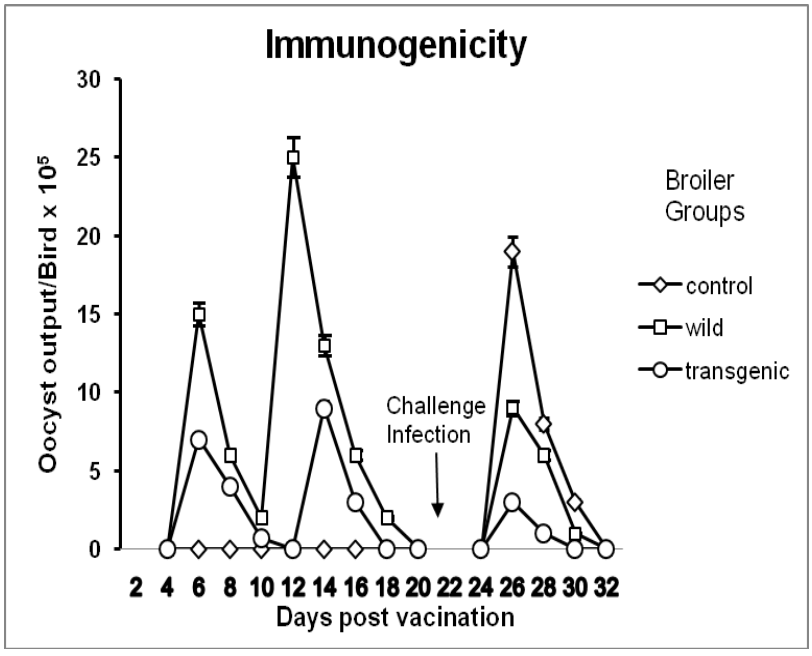


Figure 2B: Excretion of oocyst in faeces and litter samples from broilers birds every two days post vaccination with transgenic and wild type *E. mitis* and after challenge (on day 21st) with parental (wild) *E. mitis.*

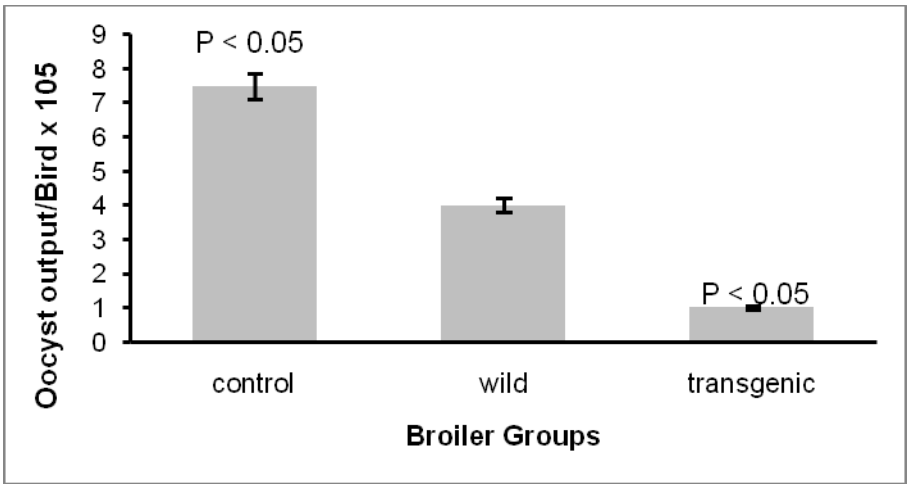


Figure 2C: Total oocyst output per bird between 4th and 8th day post challenge with *wE. mitis*.

3.5 Reduction in Peak and Cumulative Oocyst Output

Quantitative comparison of groups proved that there was a significant decrease in peak shedding intensity and cumulative oocyst production in immunized birds. Whereas unimmunized challenged birds shed at high rates, immunized birds had a reduced amount of shedding. It is found that the decline in total oocyst production after challenge is a direct indication of effective protective immunity elicited by immunization against IL-4 expressing *E. mitis*.

Table 1. Reduction in Peak and Cumulative Oocyst Output Following Challenge

Experimental Group	Peak Oocyst Shedding	Cumulative Oocyst Output	Interpretation
Unimmunized–challenged	Very high	High total oocyst production	Indicates unrestricted parasite replication
IL-4–immunized–challenged	Markedly reduced	Significantly lower total output	Demonstrates effective protective immunity

3.6 Comparison of Shedding Dynamics Between Experimental Groups

Daily shedding profiles clearly distinguished the immune status of the birds:

- **Unimmunized–challenged group:** sharp increase in oocyst output, high peak values, and prolonged shedding
- **IL-4–immunized–challenged group:** flattened shedding curves, lower peak values, and shorter shedding duration

These differences were consistent across birds and reflect suppression of parasite development rather than complete prevention of infection, a characteristic typical of effective live coccidial vaccines.

Table 2. Comparison of Oocyst Shedding Dynamics Between Experimental Groups

Parameter	Unimmunized–Challenged Group	IL-4–Immunized–Challenged Group
Onset of shedding	Rapid increase	Slower, moderated increase
Peak shedding intensity	High peak values	Low peak values
Shedding curve shape	Sharp and pronounced	Flattened
Duration of shedding	Prolonged	Shortened
Biological implication	Active parasite development	Suppressed parasite development

3.7 Clinical Correlates of Protection

Milder clinical manifestations were also coupled with the decrease in the oocyst shedding in immunized birds. Birds that were immunized continued to be normal in terms of their activity and appetite, and the intestinal upsets were minimal compared to those birds that were unimmunized and challenged. This implies that IL-4 expressing *E. mitis* protection was linked with lower pathological effect, in addition to less replication of parasites.

Table 3. Clinical Correlates Associated with Protection

Clinical Parameter	Unimmunized–Challenged Birds	IL-4–Immunized–Challenged Birds
Activity level	Reduced	Normal
Appetite	Decreased	Maintained
Intestinal disturbance	Pronounced	Minimal
Overall disease severity	Severe	Mild
Correlation with oocyst shedding	High shedding associated with pathology	Reduced shedding associated with protection

4. Discussion

The current study has shown that immunization of *Eimeria mitis* against an IL-4-expressing transgenic strain brings reproducible protective immunity against homologous wild-type infection. Protection was mainly demonstrated through a significant decrease in the reproduction of the parasite based on the fact that there was a low intensity of oocyst shedding, less cumulative oocyst output, and mild challenge clinical manifestations. These results support the idea that IL-4 expression increases the vaccine capacity of *E. mitis* without promoting an undesirable change in the attenuation/immunogenicity ratio [13].

One of the indicators of protection in coccidial vaccine testing is a decrease in oocyst shedding following challenge as opposed to total infection. In the present study, birds that were immunized continued to shed oocysts at a low level, thus proving that the challenge parasites could invade and cause development [14]. Nevertheless, replication of parasites was severely limited, and shedding curves were flattened, and the peak and total oocyst output were significantly lower. This is typical of successful live Eimeria vaccines, which act by restraining parasite proliferation by the use of immune control as opposed to sterilization immunity [15].

The reduced potency of the reproductive system in the IL-4 expressing strain during the immunization period was likely to be the contributing factor towards its desirable vaccine profile. Limited production of oocysts on the first infection guaranteed adequate antigen exposure to sensitize the immunity without causing pathological stress and environmental pollution. This regulated replication is especially beneficial to live vaccines deployment since it minimizes the danger of over-cycling of the parasite and yet permits successful immune stimulation [16].

The immunomodulatory action of IL-4 expression could be linked to the increased protection that is seen after the challenge, at least in part. It is known that IL-4 stimulates the humoral immune reaction and also regulates the inflammatory processes. The direct production of IL-4 in the intestine by the transgenic *E. mitis* strain was probably sufficient to generate a local immune environment that supported an effective immune priming response and reduced the overwhelming secretion of inflammation. This is justified by the less severe clinical symptoms in immunized birds that were challenged [8].

Significantly, immunization was effective in obtaining protection without impairing parasite viability or completion of the life cycle. The IL-4 strain that had been developed had gone through its development cycle and produced viable oocysts; this proved that genetic modification did not affect vital biological functions. This is a very high demand on live vaccine vectors, because a lack of adequate development might result in poor or unstable immune priming.

Practically, decreased fecundity, slowed and lower shedding in combination with efficient protective effect indicate the benefits of cytokine-producing Eimeria strains compared to traditional live vaccines. Conventional live vaccines are based on attenuated, or precocious, naturally occurring strains, which can still be dangerous in amplification to the environment. Conversely, genetically attenuated strains, including the IL-4 expressing *E. mitis*, implement inherent biological restrictions on replication and, at the same time, increase immune stimulation [13].

The results of the research provide evidence that Eimeria parasites might be employed as efficient vectors of immunomodulatory molecules of host origin. Incorporating the exposure of antigens with specific cytokine expression, transgenic Eimeria strains can be developed to adjust host immune response in a fashion that cannot be attained using traditional vaccination opportunities. This strategy is especially applicable to intestinal pathogens, where it is necessary to have local immune control to protect and to control the disease.

In conclusion, IL-4-expressing *Eimeria mitis* immunization resulted in a strong homologous protection with less parasite replication, fewer clinical symptoms, and controlled oocyst shedding. These attributes prove that the safety and efficacy of *E. mitis* as a live vaccine carrier are increased by the expression of IL-4. The findings have gone a long way to support the development of the next generation of vaccines against avian coccidiosis through cytokine-expressing Eimeria strains.

Conflict of Interest

The authors declare no conflict of interest.

Author`s Contribution

All authors contributed equally.

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None.

References

1. Pal, M., T. Rebuma, and T. Tolosa, *Avian coccidiosis: A major parasitic disease of poultry industry.* Int J Livest Res, 2024. **14**: p. 1-7.
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. Shakoor, A., et al., *Advancement in the development of anti-coccidial vaccines: Challenges, opportunities, and perspectives.* International Research Journal of Modernization in Engineering Technology and Science, 2021. **3**(11): p. 431-442.

4. Yu, Z., et al., *Actin depolymerizing factor-based nanomaterials: A novel strategy to enhance E. mitis-specific immunity.* Frontiers in Immunology, 2022. **13**: p. 1080630.
5. Tang, X., et al., *Genetic modification of the protozoan Eimeria tenella using the CRISPR/Cas9 system.* Veterinary Research, 2020. **51**(1): p. 41.
6. Keegan, A.D., W.J. Leonard, and J. Zhu, *Recent advances in understanding the role of IL-4 signaling.* Faculty reviews, 2021. **10**: p. 71.
7. Shen, P. and S. Fillatreau, *Antibody-independent functions of B cells: a focus on cytokines.* Nature Reviews Immunology, 2015. **15**(7): p. 441-451.
8. Fries-Craft, K., S. Schmitz-Esser, and E. Bobeck, *Dietary peptide-specific antibodies against interleukin-4 differentially alter systemic immune cell responses during Eimeria challenge with minimal impacts on the cecal microbiota.* Poultry Science, 2023. **102**(12): p. 103134.
9. Del Cacho, E., et al., *IL-17A regulates Eimeria tenella schizont maturation and migration in avian coccidiosis.* Veterinary research, 2014. **45**(1): p. 25.
10. Liu, J., et al., *Protective immunity induced by Eimeria common antigen 14–3-3 against Eimeria tenella, Eimeria acervulina and Eimeria maxima.* BMC Veterinary Research, 2018. **14**(1): p. 337.
11. El-Saadony, M.T., et al., *Alternatives to antibiotics against coccidiosis for poultry production: the relationship between immunity and coccidiosis management–a comprehensive review.* Annals of Animal Science, 2025.
12. Ahmad, R., et al., *Management and control of coccidiosis in poultry—A review.* Animal bioscience, 2023. **37**(1): p. 1.
13. 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).
14. Soutter, F., et al., *Poultry coccidiosis: design and interpretation of vaccine studies.* Frontiers in veterinary science, 2020. **7**: p. 101.
15. Savary, R., et al., *Development of a coccidiosis disease challenge model using a commercially available live oocyst vaccine.* Avian diseases, 2021. **65**(1): p. 149-158.
16. Mtshali, S. and M. Adeleke, *A review of adaptive immune responses to Eimeria tenella and Eimeria maxima challenge in chickens.* World's Poultry Science Journal, 2020. **76**(4): p. 827-841.