The Endogenous Development of *Eimeria tenella* in Chickens Injected Subcutaneously with Oocysts Protein as Initially Study of Development of Cecal Coccidiosis Killed Vaccine

The Endogenous Development of *Eimeria tenella* in Chickens Injected Subcutaneously with Oocysts Protein as Initially Study of Development of Cecal Coccidiosis Killed Vaccine

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Abstract. The study was carried out to observe the endogenous development of *E. tenella* histomorphologically in chickens subcutaneously injected with *E. tenella* oocyst protein. One day old of twenty-four broiler chickens were divided into 2 groups, each group containing twelve chicks. Group 1 was as control group injected subcutaneously on the neck with two doses: the first dose was administered at age 4th day with Freund's Complete Adjuvant (FCA) emulsified in PBS and administered booster dose was injected at 18th day of age with Freund's Incomplete Adjuvant (FICA) emulsified in PBS. Group 2 was injected subcutaneously on the neck with two doses: first dose at 4th day of age with 50 μg *E. tenella* oocyst protein emulsified in FCA and booster dose was given at 18th day of age with 50 μg oocyst protein emulsified in FICA. After 14 days of booster, the both groups were challenged orally 1 x 10^4 of virulent *E. tenella*. The assessment of endogenous development of *E. tenella* in chickens evaluated histomorphologically of cecum and oocyst production examination. Injected *E. tenella* oocyst protein chickens were challenged at 32nd day of age, demonstrated that parasite endogenous development in intestine histomorphologically appeared decreased in proliferation and suppressing oocyst production rate around 68% compared with un-injected chicken. Impaired development of endogenous parasites occurs due to protective immunity resulting from exposure to antigens so that the ability to reproduce and multiply the parasites decreases. The study results demonstrated that relatively sufficient protection against coccidia by use the *E. tenella* oocyst protein as material of cecal coccidiosis killed vaccine in broiler after challenge. *E. tenella* oocysts protein can generate protective immunity against homologous challenge through reduction of proliferation parasite and the presence of parasites disabilities.
Keywords: oocyst protein, *E. tenella*, endogenous development

1. Introduction

Coccidiosis is a parasite infection that primarily affects the intestinal tract. It is caused by a member of the genus *Eimeria* of the phylum Apicomplexa and is characterized by a complex life cycle. This

parasitic infection affects many species of mammals and birds, and is of great economic importance to livestock, especially poultry. Several species of *Eimeria* to be the cause of coccidiosis in poultry. Coccidiosis is very immunogenic, initial infection can induce strong immunity to homologous challenges [1]. The control approach through vaccination is considering more importantly, a live vaccine containing a virulent or attenuated *Eimeria* strain is available but its limited use in the poultry industry because of their high cost. Additionally, the vaccine consists of several *Eimeria* species, making it labor and cost intensive of production. Also, this type of vaccine can revert to pathogenic forms [2]. Therefore, research efforts have been invested in the development of an anticoccidial protein vaccine consisting of antigens as an alternative to live vaccines. One of protein exploration can be done to oocysts to induce protective immunity. The present study used the oocyst extract as a material of killed vaccine to protect chickens from *Eimeria* parasite. The study was carried out to inspect the endogenous development of *E. tenella* histomorphologically in chickens subcutaneously injected with *E. tenella* oocyst protein.

2. Materials and Methods

This study used twenty-four one-day-old broiler chickens as experimental animals. They were divided into 2 groups, each group containing twelve chicks. Group 1 was as control group injected subcutaneously on the neck with two doses: the first dose was administered at 4th day of age with Freund's Complete Adjuvant (FCA) emulsified in PBS and booster dose was injected at 18th day with Freund's Incomplete Adjuvant (FICA) emulsified in PBS. Group 2 was injected subcutaneously on the neck with two doses: the first administered dose at age 4th day with 50 μg antigen (*E. tenella* oocyst protein) emulsified in FCA and booster dose was injected at 18th day of age with 50 μg antigen emulsified in FICA. After two weeks of last immunization the both groups were challenged orally 1 x 104 of virulent *E. tenella*. Efficacy of protection of the use of *E. tenella* oocyst protein against homologous challenges in chickens appraised through the daily and total of oocyst production and endogenous development of *E. tenella* histomorphologically in cecum observation.

3. Results and Discussion

Injected subcutaneously birds with oocyst protein were challenged at 32nd day of age, demonstrated that oocyst protein could provide chickens with protection rate around 68%, the daily oocyst production from initial to the end passed through the faeces and also total oocysts production of *E. tenella* oocyst protein injected chickens group decrease significantly than the *E. tenella* oocyst protein uninjected chickens group (Figs. 1 and 2).

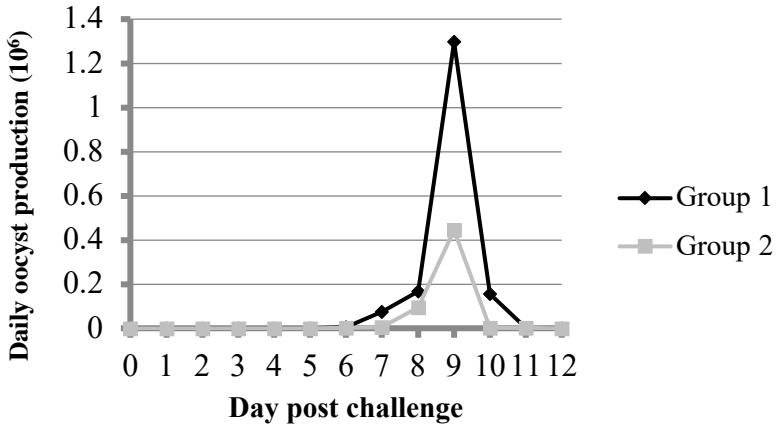

Figure 1. The comparison of pattern of daily oocyst production between *E. tenella* oocyst protein uninjected chickens group (Group 1) and *E. tenella* oocyst protein injected chickens group (Group 2). The degradation of oocyst production appeared in oocyst protein injected chickens group compared

oocyst protein uninjected chickens group exhibit potential ability oocyst protein in stimulation of protective immunity on hospes.

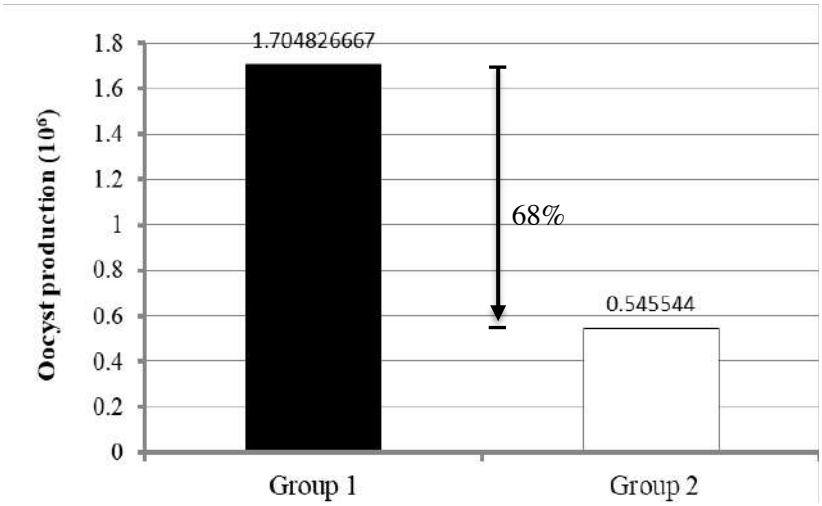

Figure 2. The comparison of oocyst production between *E. tenella* oocyst protein uninjected chickens group (Group 1) and *E. tenella* oocyst protein injected chickens group (Group 2), 68% oocyst production was suppressed in *E. tenella* oocyst protein injected chickens group.

The decrease of oocyst production in *E. tenella* oocyst protein injected chickens group emphasized by an overview of the development of parasite in predilection site as cecum histomorphologically. The few development and proliferation of parasites was seen histomorphologically in cecum (Fig. 3).

The immune response to vaccines exhibits humoral and cellular protection. The previous study suggested that specific IgG antibody responses to *E. tenella* were generated in chickens immunized with recombinant rhomboid-like proteins expressed in *E. coli* and this protein was able to elicit humoral responses and activate cell-mediated immunity in birds [3]. The study by Akhtar et al. [4] demonstrated humoral response and challenge when using supernatant of sonicated oocysts inducing strong protection because immune chicks contain high levels of antibodies to resist severe dose challenges. Sporozoite that used as protein vaccine gives 66.7% percent protection [5], while in another studies by Subramanian *et al*. [6] and Geriletu *et al*. [7] gives 60% and 77.3%, respectively percent protection when use recombinant *E. tenella* sporozoite antigen. Finally, it was found that in order to obtain the ultimate protective immunity using parasite extracts required the correct inclusion of antigens and exclusion of irrelevant antigens [8].

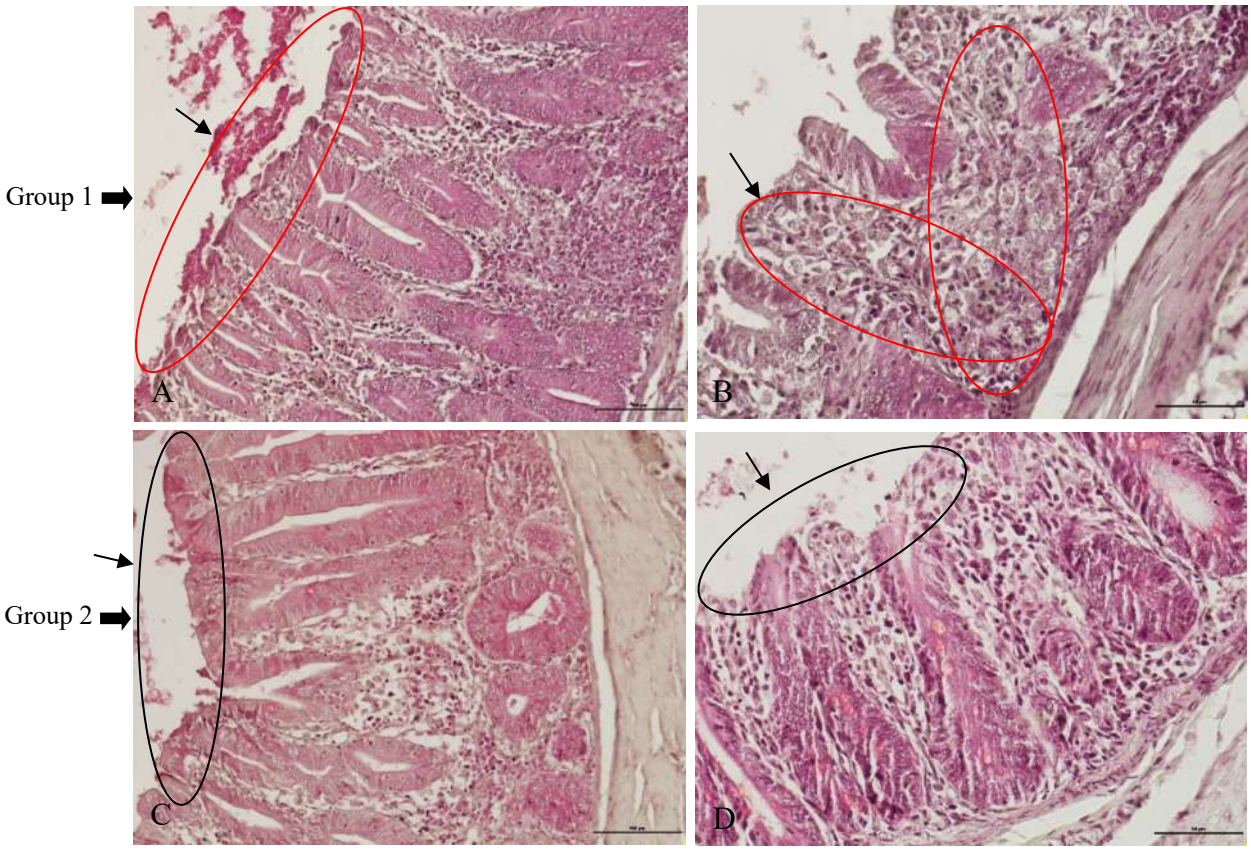

Figure 3. The *E. tenella* oocyst protein injected chickens were challenged demonstrated that parasite endogenous development in cecum appeared decreased in proliferation and suppressing to be continue development completely compared with *E. tenella* oocyst protein uninjected chickens. The development of protective immunity in *E. tenella* oocyst protein injected chickens group suppressed capacity of multiplication of parasite. A, appeared erosion of cecum mucosa; B, parasites well development; C, reduced damage of cecum mucosa; D, few parasites development. A and C (200x magnification); B and D (400x magnification). Arrow, the changes that have occurred.

4. Conclusions

The study results demonstrated that relatively sufficient protection against coccidia by use the *E. tenella* oocyst protein as material of cecal coccidiosis killed vaccine in broiler after challenge. *E. tenella* oocysts protein can generate protective immunity against homologous challenge through reduction of proliferation parasite and the presence of parasites disabilities. The further study for investigating efficacy of *E. tenella* oocysts protein in induction of protective immunity against heterologous challenge.

Acknowledgement

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1.	Valuation of The Antigenicity and Immunogenicity of *Eimeria tenella* by Reproductive Index and Histopathological Changes of Cecal Coccidiosis Virulent Live Vaccine in Broiler Chickens	2018
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6.	The conformity of diagnostic test between burrow ink test method with skin scrapping method of scabies in rabbit (Lepus domesticus)	2018
7.	The Endogenous Development of Eimeria tenella in Chickens Injected Subcutaneously with Oocysts Protein as Initially Study of Development of Cecal Coccidiosis Killed Vaccine.	2020
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