Assessment of Protective Immunity of Oocyst Protein of Wild Strain *E. tenella* on Heterologous Challenges

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Abstract

Assessment of protective immunity of wild *E. tenella* oocyst protein against heterologous challenge done in chickens. Immunization applied on 4th and 18th day of age subcutaneously with protein dose of 50 μg per chicken. Immunized chickens were challenged at 32nd day of age, demonstrated oocyst protein of *E. tenella* provide protection around 57% compared to unimmunized chickens, while parasite development, oocysts number and cecal lesion of the chickens immunized decreased significantly compared unimmunized groups. The study results demonstrated relative protection against coccidia by the use of *E. tenella* oocyst protein as vaccine in broilers.

Key words: *E. tenella*, oocyst protein, protective immunity

Coccidiosis caused by *Eimeria tenella* is a dreadful disease with a significant economic impact to the poultry industry throughout the world due to the losses from mortality and morbidity (Juárez-Estrada et al., 2021). *Eimeria* infects the epithelial cells of intestinal lining. Pathological changes may occur by this obligate intracellular pathogen, these changes differ from destruction of local mucosal barrier and underling tissues to systematic effects such as blood loss, shock syndrome and even death (del Cacho et al., 2014). The disease has been controlled by routine medication of feed with synthetic chemicals or ionophore drugs. However, the rising appearance of drug resistance and public demands for reduced drug use in poultry production have driven a dramatic change, replacing anticoccidial drugs with alternative methods, such as vaccination with either virulent or attenuated *Eimeria* oocysts (Dalloul and Lillehoj, 2006). The immunological approach is considered more important. Live vaccines containing virulent or attenuated strains of *Eimeria* are available but these types of vaccine may revert back to a pathogenic form (Sharman et al., 2010). Therefore, our research efforts have been invested in the development of anticoccidial protein vaccines composed of antigens as an alternative to live vaccines since the sporozoite is the target for protective immunity (Juárez-Estrada et al., 2021). So, the present study aims to use the oocyst extract as a vaccine to protect broilers from *E. tenella* parasite.

Materials and Methods

Twelve broiler chickens aged 1 day were divided into 2 groups, each group containing six chicks. Chicken group 1 was immunized with two doses of coccidiosis vaccine, given subcutaneously on the neck: first dose on 4th day age with Freund’s Complete Adjuvant(FCA) emulsified in PBS and booster dose on 18th day age with Freund’s Incomplete Adjuvant(FICA) emulsified in PBS. Chicken group 2 was immunized with two doses subcutaneously on the neck with two doses: first dose on 4th day age with 50 μg antigen (oocyst protein) emulsified in FCA and booster dose on 18th day age with 50 μg antigen emulsified in FICA. Two weeks after last immunization both groups were challenged orally with 1 x 10⁴ oocysts

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of virulent *E. tenella* heterologues. Protective efficacy against heterologous challenge in chickens evaluated by using protein of *E. tenella* oocyst was evaluated through oocyst production and histopathological changes examination (Mesa-Pineda *et al.*, 2021).

**Results and Discussion**

Immunized birds were challenged at 32nd day of age, demonstrated that oocyst protein could provide chickens with protection rate around 57%, oocysts number from chickens in the immunized group with oocysts protein significantly decreased than the unimmunized group (Figs. 1 and 2). Then fewer development and proliferation of parasites were seen by histopathological changes (Fig. 3).

The immune response to vaccine demonstrated humoral and cellular protection. Juárez-Estrada *et al.* (2021) reported specific IgG antibody response against *E. tenella* was generated in the chickens immunized with recombinant rhomboid like protein expressed in *E.coli* and this protein is capable of eliciting humoral

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**Fig 1.** The decrease in daily oocyst production was seen in the group of chickens that were given oocyst protein compared to the group of chickens that were not given oocyst protein, indicating the potential ability of oocyst protein to stimulate protective immunity in the host.

**Fig 2.** The protective immunity resulting from immunization using the *E. tenella* oocyst protein is approximately half that of heterologous challenge.

**Fig 3.** The development of protective immunity in the group of chickens given *E. tenella* oocyst protein suppressed the multiplication capacity of the parasite. A, the development of the parasite is clear and massive; B, some parasites multiply and others die; a and b (100x); A and B (400x); arrowheads, indicate several parasites development.
response and activating cell-mediated immunity in birds. Juárez-Estrada et al. (2021) further showed the humoral and challenge responses when the supernatant from sonicated sporulated oocyst was used which induced a strong protection as immune chicks revealed high level of antibodies to resist heavy dose of challenge. Sporozoite that used as protein vaccine gives 66.7 percent protection (Badawy and Aggour, 2006), while in another studies by Subramanian et al. (2008) and Geriletu et al. (2011) gave 60% and 77.3%, respectively with the use of recombinant E. tenella sporozoite antigen. Finally it was found that in order to get a better protective immunity by using parasite extracts, it requires the inclusion of the correct antigens and exclusion of the irrelevant ones is necessary (Wallach et al., 1994). Eimeria tenella oocysts protein can generate protective immunity against heterologous challenge through reduction of proliferation of parasites and the developmental abnormality of parasites.

Summary

Eimeria tenella oocysts protein can generate protective immunity against heterologous challenge through reduction of proliferation parasite and the developmental abnormality of parasites. Further research is needed to observe the optimum dose to produce protective immunity for both homologous and/or heterologous challenges.

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