

Original Research Article

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Detection of Cell Mediated Immune Response in Chickens Immunized with *Eimeria tenella* Sporozoites

S. Saravanan *, K.M. Palanivel, T.J. Harikrishnan and G. Selvaraju

Department of Veterinary Preventive Medicine, Veterinary College and Research Institute, Tamil Nadu Veterinary Animal Sciences University, Namakkal-637 002, Tamil Nadu, India

*Corresponding author

ABSTRACT

Keywords

Broiler chickens, *E. tenella* sporozoites, ELISA, CMI, Lesion score, Bodyweight.

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Purified *E. tenella* sporozoites were administered subcutaneously in neck region of broiler chickens in different age groups and the cell mediated immune response in terms of gamma interferons by ELISA was assessed. The mean IFN- γ concentration (pcg) in this experimental trial was significantly higher ($P < 0.01$) in T4 (226.61 ± 6.41) and lower in T1 (159.73 ± 2.37) than other groups. On 49th day of age, the concentration declined but higher in group T4 (154.65 ± 3.66) than the other groups. The mean weekly weight gain (g) after challenge was high in T4 (2342.50 ± 42.483) with a low mean lesion score (2.5 ± 0.22). A partial protective cellular immune response against caecal coccidiosis with a mean bodyweight gain, and mean lesion score superior to the unimmunized infected chickens could be observed in this study.

Introduction

The Indian poultry sector has become a major contributor to the national economy as a result of the revolutionary and scientific approaches in avian health care management. Indian poultry industry contributes nearly Rs. 9.9 billion annually to the national economy (Mohana Subramanian *et al.*, 2010). In India, broiler chicks are reared in deep litter system under farm condition which leads to frequent occurrence of the disease and face maximum economic loss due to reduced body weight gain, followed by increased feed conversion ratio (23.74%) and chemoprophylaxis (2.83%) due to coccidiosis (Bera *et al.*, 2010), and the annual losses due to coccidiosis burden was estimated to be more than \$ 800

million worldwide (Kitandu and Juranova, 2006). Coccidiosis has long been known as important in poultry, and even today, control requires significant financial expenditure (Chapman, 2003).

Chickens are susceptible to at least 9 species of coccidia and the most common species is *E. tenella*, which causes caecal coccidiosis, while *E. acervulina* and *E. maxima*, cause chronic intestinal coccidiosis (Chandrakesan *et al.*, 2009). Caecal coccidiosis is most frequent in young birds, especially at the age of four weeks (Soulsby, 1982). Currently, the increasing incidence of drug resistance to field strains of coccidian and residual effects

in the poultry meat or eggs, poses a serious problem for producers (Peek and Landman, 2011). Obviously an alternative system to control coccidiosis is by vaccination, however, use of sporulated oocysts as live vaccines has limited application (Vermeulen *et al.*, 2001) necessitating alternate immunological approaches to control the disease.

In this context, this paper presents the assessment of potency of the *E. tenella* sporozoites administered by parenteral route in broiler chickens, by enzyme linked immunosorbent assay (ELISA) by detecting cell mediated immune response and the efficacy in terms of bodyweight and lesion score in immunized broilers.

Materials and Methods

In this experimental trial, five groups of day old Cobb 400 broiler chicks were used (n=15) and purified sporozoite antigen was administered subcutaneously @ 0.1 ml per bird in the neck region, to groups T1 to T4 and T5 was kept as control. Groups, T1 and T2 were administered 10 and 20 µg of live sporozoite antigen, respectively on 2nd day of age, and T3 and T4 were administered 10 and 20 µg of live sporozoite antigen, respectively on 6th day of age by subcutaneous route in the neck region. The project proposal of this research programme to conduct these studies was duly approved by Institutional Animal Ethics Committee (IAEC) of Veterinary College and Research Institute, Namakkal.

The sera collected from experimental trial were subjected to ELISA specific to chicken gamma interferons (IFN-γ) for assessment of cell mediated immune response. The assay was carried out as per the standard protocol of the manufacturer (Cusa Biotech Ltd, China). The concentration of IFN-γ in the samples is then determined by comparing the O.D. of the

samples to the standard curve. The duplicate readings for each standard, control, and sample were averaged and subtracted from the average zero standard optical density. A standard curve was generated using the professional soft “Curve Exert 1.3” by reducing the data to generate a four parameter logistic (4-PL) curve-fit. The data were linearized by plotting the log of the IFN-γ concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. The concentrations of the test samples were derived from the following equation, Rational Function: $y = (a+bx) / (1+cx+dx^2)$.

Where, a,b,c and d from the coefficient data are variables which vary based on the fitness of standard curve derived from the test standards of each test. Statistical analysis was performed by randomized block design (Snedecor and Cochran) and analysis of variance (ANOVA) with SPSS statistical software (version 10.01). The results obtained in the experimental trial are shown in table 1 and figure 1. The potency of the sporozoite vaccine in terms of cell mediated immune response and efficacy in terms of bodyweight and lesion score after challenge with 10, 000 live *E. tenella* oocysts at 49 days of age were assessed.

Results and Discussion

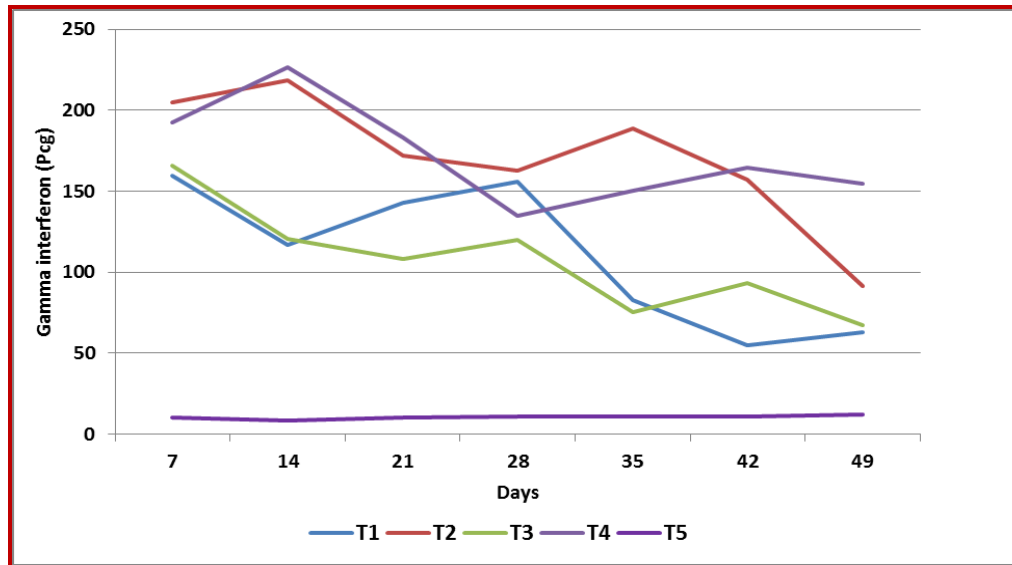
In the present study, the mean IFN-γ concentration (pcg) was significantly (P<0.01) high in T4 (226.61±6.41) followed by T2 (218.78±5.91), T3 (165.5±5.55) and T1 (159.73±2.37). High concentrations in T2 and T4 observed at 14 days of age started declining thereafter. T1 and T3 showed high concentration at 7 days of age but started declining thereafter and this early decline might be due to the low antigenic dose in the vaccine when compared to T2 and T4.

Table.1 Assessment of cell mediated immune response by IFN- γ based ELISA

Treatment Groups	Gamma interferon concentration in Pcg (Mean \pm SE)						
	7 th day	14 th day	21 st day	28 th day	35 th day	42 nd day	49 th day
T1	159.73 ^{BT} \pm 2.37	117.08 ^{BR} \pm 4.56	142.92 ^{CS} \pm 3.66	155.79 ^{CDT} \pm 4.26	82.51 ^{BQ} \pm 3.8	54.58 ^{BP} \pm 3.23	62.76 ^{BP} \pm 2.52
T2	204.57 ^{CST} \pm 10.7	218.78 ^{CT} \pm 5.91	171.81 ^{DQR} \pm 5.12	162.94 ^{CDQ} \pm 6.00	188.74 ^{DRS} \pm 2.17	156.82 ^{DQ} \pm 5.69	91.41 ^{CP} \pm 2.69
T3	165.50 ^{BRS} \pm 5.55	120.51 ^{BR} \pm 2.92	108.41 ^{BRQ} \pm 3.85	119.91 ^{BR} \pm 3.12	75.51 ^{BP} \pm 2.45	93.39 ^{CQ} \pm 4.06	67.53 ^{BP} \pm 5.25
T4	192.75 ^{CRS} \pm 3.12	226.61 ^{CST} \pm 6.41	182.95 ^{DRS} \pm 5.96	134.59 ^{BCP} \pm 2.90	150.57 ^{CQ} \pm 4.61	164.28 ^{DQ} \pm 3.65	154.65 ^{DQ} \pm 3.66
T5	10.39 ^{Apq} \pm 1.01	8.41 ^{Apq} \pm 0.51	10.33 ^{Apq} \pm 0.31	10.87 ^{Apq} \pm 0.43	10.87 ^{Apq} \pm 0.61	10.86 ^{Apq} \pm 0.49	12.33 ^{Aq} \pm 0.54

**Row-wise mean (\pm SE) with different superscript (pqr...) differ significantly (P < 0.05), mean bearing 'upper case' superscript in a row are highly significant (P < 0.01); *Column-wise mean (\pm SE) with different superscript (abc...) differ significantly (P < 0.05), mean bearing 'upper case' superscript in a column is highly significant (P < 0.01).

Fig.1 Mean ELISA IFN- γ levels in chickens of experimental trial



The mean IFN- γ concentrations between weeks within each group were significantly different ($P < 0.01$) when compared to control group. This finding is in concordance with that of Breed *et al.* (1999) and Prowse *et al.* (1991).

However, the IFN- γ concentration (pcg) on day 49 was higher in T4 (154.65 ± 3.66), followed by T2 (91.41 ± 2.69), T3 (67.53 ± 5.25) and T1 (62.76 ± 2.52) in this experimental trial. The mean IFN- γ concentration in T4 and T2 were significantly high ($P < 0.01$) than that of other vaccinated groups and this could probably be due to the higher antigenic dose administered to T4 and T2 than T1 and T3.

The mean weekly weight gain (g) after challenge, at 56 days of age was high in T4 (2342.50 ± 42.483) followed by T2 (2284.50 ± 74.407), T3 (2241.67 ± 67.333) and T1 (2214.67 ± 40.426). The weight gains of all groups were found to be superior to that of unimmunized infected T5, however, inferior to that of unimmunized uninfected T5. Similar findings were recorded by Conway *et al.*, (1990) and Kawazoe (2000). However, these weight gains were not significantly high ($P > 0.05$) when compared to unimmunized infected T5 (2278.20 ± 85.418) but significantly ($P < 0.05$) low when compared to unimmunized uninfected T5 (2518.83 ± 38.704). A low mean lesion score was observed in T4 (2.5 ± 0.22) followed by T2 (2.83 ± 0.17), T1 (3.33 ± 0.33) and T3 (3.00 ± 0.45) in comparison with unimmunized infected T5 (3.33 ± 0.33) thus indicating a partial protection ($P > 0.05$) by the sporozoites. A similar observation was recorded by Ziomko *et al.*, (2005) and Geriletu *et al.*, (2011). Interferons have been reported to be inimical to parasites, probably because of their ability to inhibit parasite development (Lillihøj and Choi, 1998), promote production of free radicals (Dimier and Bout, 1997) and

activate antibody-dependent cell-mediated cytotoxicity (Fleischer, 1980).

Hence, it is concluded that immunization of broiler chickens of less than a week old by parenteral administration with *E. tenella* specific sporozoites could result in an early but partially protective cellular immune response against caecal coccidiosis with a mean bodyweight gain, and mean lesion score superior to the unimmunized infected chickens.

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