JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2015. Vol. **9**(4), p. 3295-3298

Development of Antibodies in Broiler Chickens Immunized with Adjuvanted *Eimeria tenella* Sporozoites

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(Received: 20 April 2015; accepted: 10 June 2015)

Humoral immune response elicited by administration of purified adjuvanted *E. tenella* sporozoites by S/C in neck region of broiler chickens in different age groups was assessed by a developed indirect ELISA. The chickens of group (T2) immunized with 25 μg of antigen with Freund's Complete adjuvant (FCA) on day 2 and a booster vaccination without adjuvant on 18th day of age inducted peak IgG levels (0.616±0.022) at six weeks of age post immunization (PI) with a decline (0.394±0.012) at 7 weeks of age. A protective immune response against caecal coccidiosis could therefore be achieved by an immunization with adjuvanted *E. tenella* sporozoites in chickens of less than a week old, followed by a booster without adjuvant. Further, the performance of immunized chickens as indicated by mean weight gain (302.5±13.834g), mean lesion score (1.33±0.21) and mean oocyst output per gram (OPG) of faeces (53.84±19.09 x10^3) was found to be superior to the non- adjuvant vaccinated group and unimmunized infected control, however, the mean lesion score and OPG of faeces were inferior to that of medicated group.

Key words: *E. tenella* sporozoites, FCA adjuvant, IgG levels, weight gain, lesion score.

The efficiency of broiler production today seems to be halted by many diseases and coccidiosis amongst them poses a considerable economic loss to broiler industry, with *Eimeria tenella being o*ne of the most prevalent species in India causing caecal coccidiosis associated with reduced growth rate, poor performance and mortality in broiler chickens (KITANDU and JURANOVA, 2006). It is of great economic significance in chickens (PEEK and LANDMAN, 2011) and responsible for 6–10 per cent of all mortality in broilers (BANFIELD *et al*. 1999). The global economic losses resulting from reduction in growth rate, feed intake, and feed-conversion efficiency are estimated at 2 billion U.S. dollars annually (BANFIELD *et al*., 1999). Live oocyst vaccines currently used however have limited utility in the broiler industry in view of reduced weight gain and recycling of oocysts in the litter resulting in coccidiosis outbreaks (VERMEULEN *et al*. 2001), thus necessitating alternate immunological approaches to control the disease. Hence, this paper presents the assessment of potency of the sporozoites of *E. tenella* with and without adjuvant administered by parenteral route in broiler chickens, by a developed enzyme linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

In this experimental trial, day old hybrid strain Cobb 400 broiler chicks (n=65) were divided into treatment groups viz., T1, T2, T3, T4 and T5. Purified sporozoite antigen was administered

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subcutaneously @ 0.1 ml per bird in the neck region, to groups T1 to T4 and T5 was kept as control. T1 was administered 25 µg of live sporozoites as primary and booster doses on 2nd and 18th day of age, respectively and T2 with 25 µg of live sporozoites with Freund's complete adjuvant (FCA), as primary vaccination on 2nd day and booster vaccination without adjuvant on 18th day of age. T3 was administered live anticoccidial vaccine (Livacox-Q®) at the recommended dosage on the 2nd day of age and the group T4 was administered the anticoccidial drug (diclazuril) via feed at the recommended dosage from 2nd day of age, till the end of experiment.

The immune response of coccidian sporozoites vaccinated experimental birds were assessed by enzyme linked immuno sorbent assay (ELISA) developed as per the method recommended by CONSTANTINOIU *et al*. (2007) with slight modifications. Purified sporozoites of *Eimeria tenella* (3.6 x 10^5) were washed twice in 0.1M carbonate buffer (pH 9.5), resuspended in 1 ml of carbonate buffer and shaked by vortexing for 5 min with 0.5 mm 50 per cent glass beads. The optimal protein concentration was estimated using a standard curve derived from bovine serum albumin and purified sporozoites (*E. tenella*). The optimal dilution of coating antigen, sera and rabbit anti-chicken HRP conjugate were standardized by checker board titration and it was found that 1 µg /well of sporozoite antigen,1:50 dilution of sera and 1:5000 dilution of peroxidase conjugated rabbit anti-chicken IgG (GeNei, Bengaluru) gave optimum results. The coccidia positive serum was obtained from the birds inoculated with 3000 *Eimeria tenella* sporulated oocysts at 3 weeks of age followed by 5000 *E. tenella* sporulated oocysts at 6 weeks of age and after 21 days post inoculation, sera were collected. Negative serum samples were collected from unvaccinated control birds fed with coccidiostats. The potency of the sporozoite vaccine as assessed by IgG levels and efficacy as assessed by bodyweight, lesion score and oocysts excretion in faeces after challenge with 10,000 live *E. tenella* oocysts at 49 days of age were examined.

Statistical analysis was performed by randomized block design (Snedecor and Cochran) and analysis of variance (ANOVA two-way analysis) with SPSS statistical software (version 10.01).

RESULTS

In the experimental trial, the antibody levels in all groups assessed by ELISA were significantly higher (P<0.05) than that of control group (T5) at 7 days of age. At 49 days of age, the IgG antibody level was significantly high (P<0.01) in group T3 with a mean ELISA OD value of 0.501±0.012 followed by the groups T2 (0.394±0.012), T1 (0.310±.005) and T4 (0.057±0.005) when compared to control group (0.055±.004). The IgG antibody levels elicited in groups administered the developed and commercial vaccines were significantly higher (P<0.01) than the anticoccidial treated group and control (Figure 1). There was no significant difference in the antibody levels in the birds of T4 and T5 (P>0.05). However, peak antibody levels were observed in T2 (0.616±0.022) which were significantly different (P<0.01) from T3 (0.528±0.007), T1 (0.515±0.010) and T4 (0.067±0.001).The antibody levels of T1 and T2 rose significantly (P<0.01) on the 14th day and peaked on 42nd day of age. However, in T3, the antibody levels increased by 14 days, peaked at 28 days and declined by 35 days followed by a rise at 42 days.

The mean body weight gain ± SE (g) observed at 56th day of age post challenge in T1, T2, T3, and T4 were 256.92±13.368, 302.5±13.834, 358.250 ±8.636 and 236.15±18.761, respectively, whereas, the unimmunized uninfected and unimmunized infected group T5 had the weight gain (g) of 376.67±47.516 and 163.75±13.75, respectively. The mean lesion score in groups T1, T2, T3, T4 and unimmunized infected group T5 were found to be 1.5±0.22, 1.33±0.21, 1.17±0.17, 1.67±0.49 and 3.17±0.31, respectively. The total and mean OPG (x10^3) of faeces in T1 to T4 were found to be 67.32±25.05, 53.84±19.09, 44.64±17.4, 64.33± 24.93 respectively whereas in unimmunized

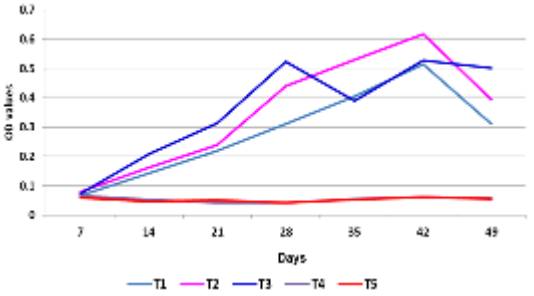

Fig. 1. Mean ELISA IgG levels in chickens of experimental trial

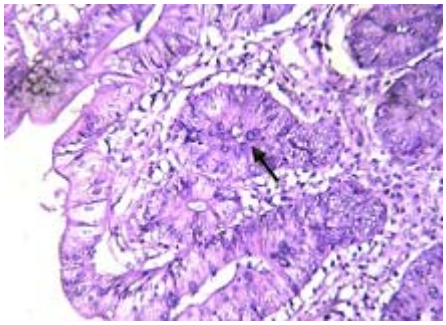

Fig. 2. Group T2 showing the mild destruction of enterocytes with developing schizonts (arrow) (H & E × 400)

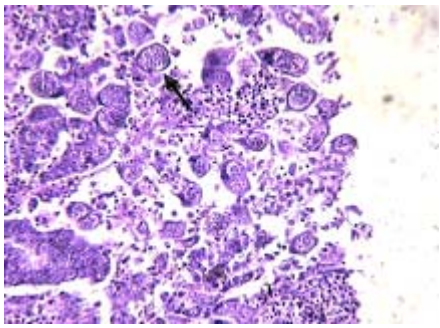

Fig. 3. Group T5 showing the large number of second generation schizonts (arrow) and infiltration of inflammatory cells (H & E x400).

infected group T5 were 1244.6, 155.58±58.89, respectively.

The microscopical changes observed at 6 day post infection (DPI) were mild destruction of the enterocytes with developing schizonts in T1, stunting of villi in T2 (Figure 2), loss of villi architecture, infiltration of inflammatory cells and presence of schizonts in T3, whereas, large number of second generation schizonts and infiltration of inflammatory cells in unimmunized infected group T5 (Figure 3).

DISCUSSION

ELISA was employed in the assessment of potency of sporozoite vaccine because of it's high sensitivity and specificity in the detection of antisporozoite antibodies of *Eimeria* species in vaccination programmes (CONSTANTINOIU *et al.* 2008). Higher antibody levels observed in the FCA adjuvanted group is in accordance with the findings of GARG *et al.* (1991) who observed protective IgG antibody levels (P<0.05) with a mean ELISA OD value of 0.245±0.028 in birds vaccinated with FCA adjuvanted *E. tenella* sporozoites than antigen alone with a similar dose and schedule and BREED *et al.* (1999) who stated that sustained immune response was maintained for a period of life time in birds vaccinated with FCA adjuvanted *E. tenella* sporozoites at zero day and booster vaccination at 21 days.

The peak and sustained IgG antibody levels (P<0.01) observed in early life of the birds in T3 than the developed vaccine might be presumably due to continuous ingestion of oocysts from the faeces or feed and water contaminated with the oocysts. VERMEULEN (2001) stated that early low level infection due to vaccination resulted in recycling of oocysts via litter, thus inducing immunity against heavy field challenge. The steady increase in IgG antibody levels within weeks of T1 and T2 might be due to two booster doses administered on day 2 and 8. The higher antibody levels observed in T2 than in T1 might be due to the potentiating effect of FCA on the immune response by *E. tenella* live sporozoites. It agrees with reports of SMITH *et al.* (1993) who observed a slow and steady increase in IgG levels by day 21 and 33.

After challenge, the mean weight gain in all the groups were significantly higher (P<0.01) than unimmunized infected T5 . However, the mean weight gain of T1, T2 and T4 were significantly lower (P<0.01) than that of unimmunized uninfected T5. However, there was no significant difference (P>0.05) between the mean weight gains of T3 and unimmunized uninfected T5 and superior performance observed after challenge in T3 could be due to the better humoral immune response than other groups. This finding is in agreement with observations of ZHANG *et al.* (2012) who reported increased weight gain in vaccinated groups than that of unimmunised infected and lower mean weight gain in medicated group after challenge.

The mean lesion score was significantly low (P<0.01) in T3 followed by T2, T1, and T4 (1.67±0.49) when compared to unimmunised infected T5. Hence, mean lesion score below 2 indicated a mild coccidiosis in groups administered with developed and commercial vaccines, and anticoccidials. This is in accordance with findings of Abbas *et al.* (2001) and GERILETU *et al.* (2011) who in their study observed a reduced mean lesion

score of 1.9 chickens vaccinated with FCA adjuvanted *E. tenella* sporozoite vaccine than antigen alone when compared with positive control.

There was no significant difference between the mean OPG of all groups, however, the mean OPG of T1 and T2 were significantly lower ($P<0.01$) than that of unimmunized infected T5. This finding is in agreement with ZIOMKO *et al.* (2005) who recorded 41 per cent reduction in oocyst output in Coxabic® vaccinated chickens, however, 23.5 per cent reduction in mean OPG of *E. tenella* sporozoites vaccinated broilers.

In histopathology, the caeca of group T1 and T2 revealed mild destruction of enterocytes with stray developing schizonts when compared to T3 and T4 and these corroborate LILIC *et al.* (2009) and PATRA *et al.* (2010).

CONCLUSION

It is concluded that immunization with specific sporozoites adjuvanted with FCA by parenteral administration in broiler chickens resulted in an early but protective humoral immune response (IgG) better than non- adjuvanted group and unimmunized infected control, however lower than commercial vaccine administered group against caecal coccidiosis. The performance of the adjuvant vaccinated group in terms of mean bodyweight gain, mean lesion score and faecal oocyst output was superior to that of non- adjuvant vaccinated group and unimmunized infected control, however it's mean bodyweight gain inferior to that of commercial vaccine administered group and it's mean lesion score and OPG of faeces inferior to that of anticoccidial administered group.

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