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Prophylactic Efficacies of the Locally Prepared *Eimeria tenella* Vaccine in Broiler Chicken

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ABSTRACT

Eimeria tenella is one of the important species as it causes caecal coccidiosis in chickens. Coccidiosis is believed to be controlled effectively using vaccines. The present study investigated the prophylactic efficacy of two locally prepared vaccines against caecal coccidiosis in broiler chicken. Broiler chicks (n = 80) were divided in four different groups A, B, C and D (n = 20 birds/group), reared as healthy control, infected control, vaccinated only once with formalin inactivated *Eimeria tenella* (FEV-48) vaccine on day 10 of age with 1000 oocysts orally and vaccinated with irradiated *Eimeria tenella* (IEV) on day 3 and 10 of age with 1000 oocysts orally, respectively. Birds were infected orally except the negative control group with 20,000 sporulated oocysts of *E. tenella* on the day 16 and 26. The birds were weighed weekly. Feces were collected on day 19, 23, 26, 29, 33 and 36 to determine oocysts per gram of feces (OPG). Result showed that the broilers of groups C and D weighed more compared to group B. The OPG count reduced significantly ($p < 0.05$) in the groups C and D compared to the group B. These promising results of using locally prepared vaccines need evaluation by humoral and cellular responses.

***Key Words*:** Coccidiosis, *Eimeria tenella,* Vaccine, Weight gain

INTRODUCTION

Coccidiosis is a worldwide devastating disease caused by apicomplexan parasites of the genus *Eimeria* (Williams et al., 1999). Avian coccidiosis has the greatest economic impact on poultry production in terms of costs of prophylactic in-feed medication for broilers and broiler-breeders, alternative treatments if the medications fail, and losses due to mortality, morbidity and poor feed conversion of birds that survive outbreaks (Williams, 1998).

Eimeria tenella is the most important species, as it causes caecal coccidiosis in chickens (Shirley, 2000). It primarily invades and resides in the epithelial linings of caeca of exposed chickens (Vervelde et al., 1995; Yun et al., 2000). Chemoprophylaxis and anticoccidial feed additives have been used to control the disease but in turn have complicated the problem with the emergence of drug resistance (Stephen et al., 1997). Keeping in view the drug resistance and other problems, use of vaccines against coccidiosis has been advocated (Anwar et al., 2008).

Vaccination is effective and safe alternate to control coccidiosis. Several commercial vaccines are being used to control coccidiosis in different countries (Crouch et al., 2003; Danforth, 1998), but there is a risk of introducing unwanted *Eimeria* species in the environment as there is a regional variation in the antigenicity of coccidial strains used in the vaccines (Fitz-Coy, 1992; Martin et al., 1997). So far two imported vaccines, namely; Immucox and Coccivac (Shirley et al., 1995) are commercially available in Pakistan. The trial of locally

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prepared vaccine (Eimeria vaccine) has also been conducted (Hashmi et al., 1994) to control the said disease in birds. Locally prepared vaccine has been found to be equally effective compared with imported vaccines (Hashmi et al., 1994). The present study, therefore, was designed to investigate the efficacy of two locally prepared anticoccidal vaccines (Formalinized Oocysts; Irradiated Oocysts) on weight gain and oocyst counts (OPG) in broiler chicken.

MATERIALS AND METHODS

Experimental Animals

Eighty day-old broiler chicks (Hubbard) were purchased from a local hatchery. The chicks were reared under standard hygienic conditions at the University of Veterinary and Animal Sciences, Lahore on deep litter system. Birds were fed ad libitum a commercial diet free of coccidiostat (Kashmir Feed Mill Limited, Lahore). The birds were vaccinated against New Castle Disease (ND) on day 1 and 21.

Collection and Sporulation of Oocysts

Oocysts for experimental infection were isolated from the caeca of naturally infected chickens brought to the University Diagnostic Laboratory. The oocytes were sporulated as described earlier (Rashid et al., 2009).

Vaccine Preparation

Formalin treated *E. tenella* vaccine, namely FEV-48, was prepared by a method as described elsewhere (Hashmi et al., 1994). Briefly, the sporulated oocysts were treated with 3 % formalin for 48 hours. The oocysts were washed properly and stored in normal saline solution. Likewise, the irradiated vaccine (IEV vaccine) was prepared by exposing the sporulated oocysts to X-rays @ 40 MAS/50KV for 0.16 second from distance of 100 cm.

Experimental Design

On day 3, the birds were randomly divided into four groups (A, B, C and D), each containing 20 birds. Group A was reared as non-infected, non-medicated control group. Group B was kept as infected, non-medicated control group. The birds of group C were vaccinated once orally with FEV-48 on day 10 with 1,000 oocysts. The group D of birds was vaccinated orally with IEV on day 3 and 10 with 1,000 oocysts. All birds except of group A were experimentally infected orally with 20,000 sporulated oocysts of *E. tenella* on day 16 and 26. The efficacy of each vaccine was assessed in terms of weight gain and reduction in the ova of oocytes.

The birds were weighed on day 19, 23, 26, 29, 33 and 36 to record the weight gain. Oocysts per gram (OPG) counts of feces was carried out on day 3, 7 and 10 post-infection using a McMaster Egg Counting technique (Anonymous, 1986).

Statistical Analysis

Comparisons of quantitative values in the different groups were performed using the ANOVA test, after checking the homogeneity of variances. Comparisons between groups were performed by Tukey test, considered significant at < 5% ($p < 0.05$). All statistical estimates were made using the statistical package GraphPad Prism5.0® (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS AND DISCUSSION

The current study was conducted in broiler chicks to evaluate the prophylactic efficacy of locally prepared formalin treated or irradiated *Eimeria tenella* vaccines.

Coccidiosis in chickens can be controlled effectively through the use of vaccination strategy. However, there is likelihood of introducing unwanted *Eimeria* species into the environment by the use of imported vaccines as there is a regional variation in

the antigenicity of coccidial strains (Fitz-Coy, 1992; Martin et al., 1997). Previously our laboratory tested a locally prepared anticoccidial vaccine against Eimeria in chickens and found it to be equally effective compared with imported vaccines (Hashmi et al., 1994).

The prophylactic effect of FEV-48 and IEV vaccines on body weight gain is shown in Figure 1. Maximum mean weight of non-infected healthy chicks (group A) was 1,064g and the mean weight of other groups remained under this limit. The Group B (infected control) birds showed only 67.80% body weight gain. The birds of Groups C (FEV-48-vaccinated) and D (IEV-vaccinated) showed 70.2% and 70.3% increase in body weight gain respectively at the end of the experiment.

The prophylactic effect of FEV-48 and IEV vaccines on OPG counts is shown in Figure 2. In Group A, no oocyte was detected. In group B, OPG ranged between 5,000 to 395,000. In Group C, the infection remained under 1,000 OPG counts up to day 24, rose to the peak of 38,000 on day 26 and declined to 7,500 on day 29 which again reached the peak of 125000 on day 33 that was due to additive effect of challenge infection. On day 36, the OPG count declined to 53,000 showing the development of immunity. The OPG ranged between 0 and 1,25,000 in this group. However, up to day 29 the infection remained at moderate level and no mortality occurred in this group. The significant ($p < 0.05$) reduction of OPG was observed in the group C compared to the group B. The results are substantiated with Hashmi et al., (1994), Rashid et al., (1998), Ashraf (1999). They used formalin treated locally prepared vaccine on day 3 and 10. They claimed that the vaccine showed greater resistance to primary and challenge infections as compared to other remedial measures. In the present study, if the vaccine could have been administered on day 3 as well, the results would have been far better.

The birds of group D were orally administered with locally prepared IEV vaccine on day 3 and 10. The OPG counts in this group ranged from 0 to 37,000. On day 23, the OPG count declined to 0 which again rose to 37,000. On day 36, it returned to 18,000. The significant ($p < 0.05$) reduction in OPG count was observed in the group D compared to the group B (positive control). The finding of the present study is closely correlated with Afzal (2001) who reported that irradiated *Eimeria* Vaccine (IEV) was quite efficient in controlling caecal coccidiosis in broilers. Blake et al., (2005) reported that sporulated Eimerial oocysts were consistently capable of stimulating immunity in broilers when are experimentally infected with 100 oocysts of a homologous strain (Blake et al., 2005).

In conclusion, both locally prepared vaccines proved effective in reducing the OPG count. However, the efficacy of these vaccines may be further studied to evaluate their impact on humoral and cellular immune responses.

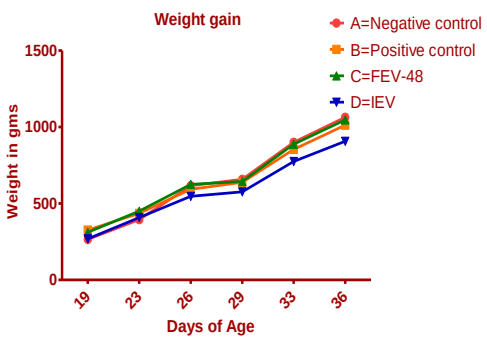

Figure 1 Body weight gain in broilers on different days

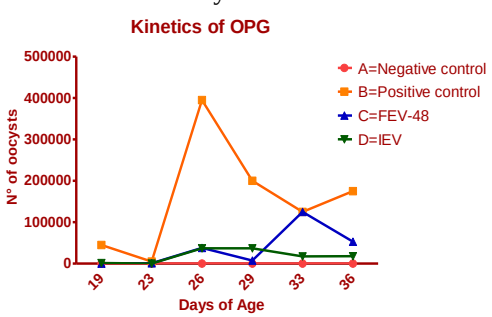

Figure 2 OPG count of Eimeria in broilers

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