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Immunization of Chicken with Live *Eimeria tenella* Sporulated Oocysts for Control of Caecal Coccidiosis

Shafiya Imtiaz Rafiqi, Rajat Garg*, Reena K.K, Hira Ram and P.S. Banerjee

Division of Parasitology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P., INDIA

**Corresponding author: R Garg; Email: rajatgarg_2000@yahoo.com*

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ABSTRACT

Eimeria tenella is the most pathogenic and one of the commonest species of *Eimeria* infecting broiler chickens raised under deep litter system throughout the world, causing caecal coccidiosis and incurring huge economic losses to the poultry industry. In the present study, the immunization potential of live *Eimeria tenella* oocysts was evaluated following homologous oocyst challenge in broilers. The birds were immunized at seven and 21 days of age orally with 1000 live sporulated oocyst of *Eimeria tenella* and challenged with homologous strain of parasite on 28 days of age. The immunization potential was evaluated in terms of relative weight gain, caecal lesion scoring, oocyst output and the anti-coccidial index (ACI). The results revealed that immunization with live oocysts of *Eimeria tenella* resulted in significant relative weight gain (82.47%), reduction in oocyst output (93.74%) and ACI of 161.47, indicating that oral immunization of chickens against *E. tenella* was effective in preventing the clinical disease and decreasing the oocyst burden in poultry farms.

Keywords: *Eimeria tenella*, live oocyst immunization, chickens

Poultry coccidiosis, caused by protozoan parasites belonging to the genus *Eimeria*, is cosmopolitan in distribution and is associated with global economic losses in excess of two billion Euros (Peek and Landman, 2011). Out of the seven widely recognized species of *Eimeria* infecting the domestic fowl, *Eimeria tenella* is the most pathogenic one. Losses are incurred due to mortality, malabsorption, inefficient feed utilization, impaired growth rate of broilers and reduced egg production in layers (Lillihoj *et al*., 2004).

Control of coccidiosis in poultry is achieved by strict managemental practices combined with in-feed anticoccidial drugs or vaccination with live parasites. Resistance to anticoccidial drugs has been recognized for decades and regarded as ubiquitous (Chapman, 1997). In India, varying degrees of efficacy of anticoccidials has been reported (Yadav and Gupta, 2001; Gautam and Gupta, 2004). Resistance to sulfaquinoxaline, nitrofurazone plus furazolidone, amprolium, clopidol, nicarbazin, sodium sulphadimethyl pyrimidine and maduramicin in various field isolates of *Eimeria* sp. has been reported from north India (Rana, 1993; Agarwal *et al.,* 2013).

Since *Eimeria* spp. are highly immunogenic and can stimulate solid immunity to homologous challenge, immunological control can serve as a practical alternative to indiscriminate use of anticoccidials. Based on this concept, a number of live or live attenuated vaccines *viz.* Coccivac, Immucox, Paracox, Livacox etc. have been marketed globally, but no commercial vaccine is available for use in India. Hence, the present study was undertaken to evaluate the anticoccidial efficacy of live *E. tenella* oocyst immunization against homologous oocyst challenge in broiler chicks.

MATERIALS AND METHODS

Experimental chickens

Seventy five day old unimmunized broiler chicks (Caribro strain) were procured from CARI, Izatnagar and reared

under coccidia-free environment in brooders with raised wire netting floor inside a well ventilated room. All the chicks were fed coccidiostat free feed and water *ad libtum*. Chickens were randomly assigned to three groups, twenty five birds in each namely oocyst immunized, unimmunized challenged and unimmunized unchallenged.

Eimeria tenella oocysts

Clonal line of *Eimeria tenella* (Indian isolate), derived by single sporocyst inoculation of chickens, was used in the study (Kundu, 2014). The sporulated oocysts of this strain were propagated in 3-4 week old chicks before the start of experiment and used for immunization and challenge studies.

Immunization and homologous sporulated oocyst challenge

One-day old broiler chicks were divided into three groups of 25 chicks/group. The chicks of group I (OI group) were orally immunized with 1000 sporulated oocysts of *Eimeria tenella* at 7 days of age and a booster dose of 1000 sporulated oocysts was given at 21 days of age. The chicks of group II (UIC group) were kept as unimmunized-challenged controls, while those of group III (UNUC group) were kept as unimmunized-unchallenged controls. At 28th day of age, the chicks of group I and II were orally challenged with 1x10^4 sporulated oocysts of *E. tenella*. Extreme care was taken to avoid adventitious exposure of chicks to coccidia during immunization period and faecal droppings from each group were periodically examined for the presence of oocysts until day 5 post-challenge.

Relative body weight gain

Body weight of chickens (n= 10/group) was measured on 0 day and 12 day post-challenge. The body weight of birds at 12 day post-challenge was subtracted from the body weight of the chicks at the time of challenge (0 day post-challenge) to determine the body weight gain. Percent relative weight gain (RWG%) was calculated as per following formula:

RWG% = (Weight gain of the OI group)/ (Weight gain of the UNUC group) × 100

Caecal lesion scoring

The severity of infection was assessed by lesion scoring technique described by Johnson and Reid (1970). For this purpose, ten birds from each group were sacrificed by cervical dislocation on day 6 post challenge and their caeca were collected for lesion scoring. Any mortality in challenged birds was also recorded. The following pattern of scoring was used for *E. tenella*: 0- No gross lesions, +1- Very few scattered petechiae on the caecal wall; no thickening of the caecal walls; normal caecal contents present, +2- Lesions more numerous with noticeable blood in the caecal contents; caecal wall somewhat thickened; normal caecal contents present,+3- Large amounts of blood or caecal cores present, ceacal walls greatly thickened: little, if any, faecal contents in the caeca,+4- Caecal wall greatly distended with blood or large caseous cores; faecal debris lacking or included in cores. Dead birds scored as +4.

Faecal oocyst count

Complete faecal droppings from each group were collected separately between 5th and 11th day post-challenge, weighed and oocysts per gram of faeces (OPG) were determined using McMaster counting technique (Lillehoj and Ruff, 1987). An average of three OPG counts per group was taken. Based on the total oocyst output per bird [(average OPG per group × total weight of faecal droppings per group)/ total number of birds per group], percent oocyst reduction was calculated as follows:

(Number of oocysts from the challenged control group - Number of oocysts from the vaccinated group)/ Number of oocysts from the challenged control group × 100.

Anticoccidial index

Protective efficacy of the oocyst immunization against *E. tenella* infection was evaluated on the basis of anticoccidial index (ACI) which was calculated by using the formula as described by Li *et al.*, 2004.

ACI = (Relative weight gain + Survival rate) - (Lesion score value + Oocyst value).

The protective efficacies of different recombinant proteins as determined in terms of ACI were classified as very effective (ACI: ≥180), effective (ACI: 160-179),

moderately effective (ACI: 120-161), not effective (ACI: ≤120).

Statistical analysis

The data generated were analyzed by one way analysis of variance(ANOVA) followed by the Duncan's multiple range using SPSS 16.0 for windows (SPSS Inc., Chicago, IL). Differences between groups were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

The efficacy of immunization was evaluated on the basis of body weight gain, lesion score and oocyst shedding. No mortality was observed in any experimental groups.

Body weight gain

Chicken of unimmunized challenged group (group II) exhibited reduced average weight gain (560.01±63.36g) as compared to oocyst immunized (661.7±56.58g) and unimmunized unchallenged control group (802.33±11.51g). The relative weight gain of oocyst immunized group was 82.47% as compared to 69.79% of unimmunized challenged group indicating impaired weight gain in unimmunized group (Table 1).

Caecal lesion scores

The average caecal lesion score in unimmunized challenged group (group II) was 2.6±0.40 and that of oocyst immunized group (group I) was 1.6±0.24 (Table 1). No caecal lesions were seen in unimmunized unchallenged control group (group III).

Faecal oocyst counts per bird

The mean OPG in oocyct immunized birds was $0.14\pm0.06\times10^4$, while in unimmunized challenged birds it was $2.70\pm1.2\times10^4$. The total oocyst output in oocyst immunized group was $1.74\pm 0.24\times10^6$ as compared to $27.8\pm1.3\times10^6$ in unimmunized challenge group (Table 1). Compared to unimmunized group, the oocyst shedding in oocyst immunized group was significantly ($p\leq0.05$) reduced by 93.74%.

Anticoccidial index

On the basis of lesion score, relative weight gain and reduction in oocyst shedding, the ACI of oocyst immunized group was calculated as 161.47, indicating that oral immunization of chickens against *E. tenella* was effective in preventing the clinical disease.

Demand for green food products and anxiety about drug residues has led to upsurge in use of vaccine against coccidiosis. The use of live vaccines for the control of coccidiosis in broilers or layers is well established all over the world but unavailability of anticoccidial vaccines in India puts forth limited options before poultry producers. Live attenuated or non-attenuated coccidiosis vaccines may offer a realistic long term solution to the coccidiosis problem (Vermeulen *et al*., 2001), until subunit vaccines are commercialized on large scale, particularly in India. However, successful use of live oocyst attenuated and non-attenuated vaccines is dependent on housing management that balance oocyst cycling with moderate numbers of infective oocysts. Among various *Eimeria* species, *Eimeria tenella* is highly pathogenic and the most prevalent species infecting chickens in India (Prakashbabu *et al*., 2017). The present study is an effort to evaluate the

Table 1: Protective efficacy of oral immunization with live *Eimeria tenella* oocysts against homologous challenge in broiler chicks

Groups	Average body wt. gain (gm)	Relative body wt. gain (%)	Average caecal lesion score	Oocysts output per bird (× 10^6)	Oocyst reduction (%)	Anti-coccidial index
Gp. I	661.7±56.58^a	82.47	1.6±**0.24**	1.74±**0.23**^{a}	93.74	161.47
Gp. II	560.01±63.36^b	69.79	2.6±**0.40**	27.8±**1.005**^{b}	—	—
Gp. III	802.33±11.51^c	100	—	—	—	—

Values are expressed as mean ± SE. Means in the same column with different superscripts were significantly different between treatment groups ($p < 0.05$)

protective efficacy of live oocyst immunization against *Eimeria tenella* oocyst challenge.

Our study demonstrated immunizing broiler chicks with oral infection of 1000 sporulated oocyst of *Eimeria tenella* on 7 and 21 days of age resulted in limiting the pathogenicity and significant oocyst reduction upon homologous challenge with 1×10^4 sporulated oocysts on day 28 of age. Since, the oocyst is environmentally resilient and can survive exogenously for long time, decreased oocyst burden in the litter can prevent potential contamination among successive stocks. Acquired immunity can be generated for a homologous species of *Eimeria*, similar to that elicited by a trickle infection, with limited or no pathogenesis (McDonald and Shirley, 2009).

In the present study, relative weight gain was higher in vaccinated as compared to unvaccinated birds. Danforth (1998) also demonstrated that most broiler breeds exhibited a transient slight drop in weight gain after vaccination, but recovered quickly and compensated for the loss after 3 or 4 weeks. In another series of field experiments it was shown that vaccinated flocks had higher feed conversion rates than non-vaccinated but medicated flocks (Williams *et al*., 1999).

One of the major pitfalls of live oocyst vaccination is immunovariation. Comparison of the cross-protective immunizing capacity of different strains of live *E. tenella* parasites revealed significant strain-specific variation and some lack of heterologous immunoprotection, although the breakthrough in parasite replication was at a level unlikely to lead to occurrence of clinical disease (McDonald *et al*., 1986; Blake *et al*., 2015). Genetic and antigenic diversity has been described for *E. tenella* (Awad *et al*., 2013; Blake *et al.,* 2015; Kumar *et al.,* 2015; Clark *et al.* 2016), but it is at a much lower level than reported for *E. maxima* and *E. acervulina* (Blake *et al.*, 2012).

Although there are problems in the implementation of viable potentially virulent oocyst based vaccines in broilers, interest in their use to control coccidiosis will continue to grow as long as any subunit vaccine comes into the market particularly in a developing country like India.

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