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# Evaluation of Kadaknath Chicken for Coccidial Resistance by Oocyst Count, Lesion Scoring and Oocyst Index in *Eimeria Tenella* Infection

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**ABSTRACT**

The aim of present investigation was to study the virulence of coccidiosis in Kadaknath birds, experimentally infected with *E. tenella* by OPG count, oocyst index and caecal lesions scoring. Sixty one-day old unsexed Kadaknath birds were randomly divided into a control, T1 and T2 groups comprising twenty chicks under each group. At d 21 of age, birds of T1 and T2 groups were individually inoculated with 10,000 and 20,000 of sporulated oocysts of *E. tenella* and from the d 5 to 9 pi, faecal droppings were collected for counting OPG. Also at d 4, 7 and 14 after *E. tenella* infection, 2 chicks from each challenge groups were euthanized and caecal lesions and oocyst index were scored. The results indicated that in both T1 and T2 challenge group, there was an increasing trend of OPG upto d 7 pi and the peak level of OPG was found at d 7 pi. Mean OPG was significantly ($P<0.05$) higher in T2 at d 6, 7 and 8 pi than T1. Mean caecal lesion score, oocyst index and OPG count were found to be maximum on d 7 pi for T1 and T2 groups. Lesions score was non significant at 4, 7 and 14 pi. However, oocyst index was significant ($P<0.05$) at d 7 pi. No mortality was observed in any of the treatment groups. As Kadaknath birds shed less oocyst and had lower lesion scores and oocyst index, hence, it can be concluded that this breed is less susceptible to coccidial infection.

**Keywords:** Kadaknath, coccidiosis, OPG, lesions score, oocyst index

Genetic resistance to disease is an important part of comprehensive poultry health program. Genetic selection to increase immune responsiveness of disease resistance can make permanent improvements in fitness and also enhance vaccine effectiveness. Chickens can harbor a number of different types of pathogens like viruses, bacteria and parasites, all of them may cause major diseases in poultry (Lamont, 1998). Coccidiosis is an important and major parasitic disease of poultry caused by apicomplexan protozoa belonging to at least seven different species of *Eimeria.* The most common and pathogenic species that affects the poultry industry is *E. tenella*, resulting in 100% morbidity and a high mortality due to extensive damage of digestive tract (Shirley and Lillehoj, 2012).

In India, coccidiosis is a serious problem and is one of the biggest causes of economic losses in poultry. The mortality of birds is usually attributed to both severe caecal haemorrhage and excessive blood loss alone or coupled with toxic factors and bacterial products. However, the continuous and indiscriminate use of chemicals worldwide may limit the usefulness of anticoccidial drugs as there is always an apprehension of emergence of drug resistant strains of *Eimeria* species. Hence, the disease continues to be the serious and economically important health problem of broiler chicks for poultry farmers (Bera *et al.*, 2010; Nikam *et al.*, 2012). It has been reported that one parameter measured from chickens with coccidiosis do not truly reflect the genetic resistance of an individual

(Caron *et al.*, 1997). Therefore, caecal lesion score, oocyst index and fecal oocyst shedding in terms of oocyst per gram (OPG) are the most commonly measured parameters for the evaluation of genetic resistance or susceptibility to coccidiosis (Pinard-van der Laan *et al.*, 1998; Shirley and Lillehoj, 2012). There is a dearth of literature available on the coccidial resistance in Kadaknath breed of chicken. Therefore, the present study was planned to study genetic differences in susceptibility during an experimental coccidial infection by OPG counting, lesion scoring and oocyst indexing in Kadaknath breeds of chicken.

## MATERIALS AND METHODS

### Maintenance of chicks

The study was carried out on sixty day old Kadaknath chicks maintained in the brooder batteries (cages) kept inside a well ventilated room in the college experimental poultry shed unit under proper coccidian free conditions. Chicks were randomly divided into a control (C) group and two treatment groups ($T_1$ and $T_2$) comprising twenty chicks under each group. During the entire experimental periods there was continuous lighting. The basal diet used did not contain growth promoters or coccidiostats and were given water *ad libitum* throughout the whole experiment.

### Fields isolates of *Eimeria tenella*

Field isolates of *Eimeria spp.* were collected from Central Poultry Diagnostic Laboratory (Phoenix Group) 1333/1, Narmada Road, Jabalpur and unorganized farms in and around Jabalpur. The oocysts of *E. tenella* were collected from the caecum of infected broiler chicks. The *E. tenella* infection was identified by the appearance of predominant caecal lesions and presence of characteristic schizonts and gametocytes in the freshly prepared smears of caecal mucosa. After proper identification, the oocysts were collected from the caeca of these birds and were processed to have inoculums of *E. tenella* in the laboratory as per the method described by Davies *et al.* (1963) with some modifications.

### Preparation of *E. tenella* inoculum

The *E. tenella* inoculum for the different isolates was prepared separately in three different stages i.e. harvesting of oocysts, sporulation of oocysts and storage of inoculums. The caecal contents of infected birds were homogenized in 2.5 per cent potassium dichromate solution and the homogenate was filtered through muslin cloth to remove large debris. Afterwards, the filtrate was sieved sequentially through three sieves of different mesh sizes i.e. 40, 80 and 100 meshes per linear inch, respectively. The sieved material was then centrifuged in 50 ml plastic centrifuge tubes at 1500 rpm for 2 minutes. The pellet of centrifugate was subjected to floatation technique in saturated sodium chloride solution to obtain the oocysts. The surface layer in each tube containing oocysts was then pipetted out into a large volume of tap water to dilute the salt solution and the oocysts were allowed to sediment overnight. The supernatant was discarded and the sediment was centrifuged again to remove water. The sediment of oocysts was resuspended in 2.5 per cent solution of potassium dichromate for sporulation. The sporulation was carried out by distributing the oocysts suspension in 2.5 per cent potassium dichromate solution in shallow layers (3-5 mm) in large petri plates (6 inch in diameter) which were then placed in a BOD incubator at $29\pm1^0$C. The forced aeration of the suspension was carried twice daily in order to prevent the oocysts under sporulation from drying. The 2.5 per cent potassium dichromate solution was added repeatedly till the sporulation of oocysts was completed. The oocysts were regularly examined daily for their sporulation upto 52 hrs. The sporulated oocysts were transferred into conical flasks (250 ml) having sufficient postassium dichromate solution (2.5%). These flasks were then labeled and stored at $4^0$C till further use. The number of sporulated oocysts in the suspension was estimated using McMaster counting chamber and the volume was adjusted to contain the 10,000 and 20,000 sporulated oocysts/ml of suspension.

### Coccidial challenge with sporulated oocyst of *E. tenella*

Faecal samples of all the birds from each group were examined to confirm the absence of *Eimeria* species, before the coccidial challenge. Group $T_1$ and $T_2$ were challenged by gavaging 10,000 and 20,000 sporulated oocyst, respectively to each bird on 21st days of age. The control group was given 1 ml of Hanks Balanced Salt Solution (HBSS). The faeces voided by the birds were collected daily from day (d) 5 to 9 post infection (pi) and was taken to the laboratory for estimation of OPG

by following the method of Davies *et al*. (1963), using McMaster counting chamber. On the other hand, at d 4, 7 and 14 pi, two birds were randomly selected from each group and were sacrificed for evaluation of lesion score and oocyst index based on macroscopic visible lesions caused by *E. tenella*. The caeca of sacrificed birds were removed at each interval and used for lesion scoring as per standard procedure (Johnson and Reid, 1970). An oocyst index was determined by microscopic examination from each segment of caeca for birds sacrificed for lesion score at d 4, 7 and 14 pi for counting of oocysts per field as per the standard method (Hilbrich, 1978). For statistical analysis, data were analyzed by Hierarchical design of ANOVA using MSTAT-C software. When significant differences among means were found, data were analyzed by Post-hoc and compared by Duncan's Multiple Range test (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

### OPG count at day 5 to 9 post infection

The mean OPG recorded in two challenged groups ($T_1$ and $T_2$) at d 5 to 9 pi in Kadaknath have been presented in table 1. Mean OPG count differed significantly ($p<0.05$) between T1 and T2 at d 6 pi; while at remaining dpi, it did not differ significantly (P>0.05). The highest mean OPG count was observed on d 7 pi under each dosage of coccidial infection. Mean OPG count showed increasing trend till d 7 pi and then started declining and reached minimum at d 9 pi. Our results on the association of OPG with dosage of coccidial challenge are corroboratory to the finding of earlier workers. Gabriel *et al.* (2003) reported significantly higher mean OPG in infected birds of WLH chicken. Further, findings of increasing trend in OPG upto d 7 pi and declining afterward were also reported by Kostadinovic *et al.* (2012) in chicken when challenged with 20,000 oocyst of *E. tenella*. Likewise Jatau *et al.* (2014) and Shojaei (2014) have reported significantly higher OPG count in broiler strain of chicken as compared the present findings. The reason for observing peak OPG at d 7 pi in present findings can be correlated with post infection symptoms and clinical signs of normal life cycle of *Eimeria* species in poultry (Elmusharaf *et al.*, 2010; Velker, 2011; Clark and Blake, 2012).

**Table 1.** Mean (±SE) OPG ($10^6$) at day 5 to 9 post infection in Kadaknath chicken

| Treatment / Interval | $T_1$ | $T_2$ | Overall |
|---|---|---|---|
| Day 5 | 3392.0$^a$ ± 131.60 | 3592.0$^a$ ± 144.67 | 3492.0 ± 100.60 |
| Day 6 | 4967.0$^b$ ± 156.84 | 7053.0$^a$ ± 204.14 | 6010.0 ± 219.38 |
| Day 7 | 6175.0$^b$ ± 194.06 | 9489.0$^a$ ± 174.61 | 7832.0 ± 309.78 |
| Day 8 | 5197.0$^b$ ± 192.57 | 6883.0$^a$ ± 168.54 | 6040.0 ± 199.18 |
| Day 9 | 3597.0$^a$ ± 247.84 | 4431.0$^a$ ± 186.61 | 4010.0$^a$ ± 174.25 |

*Values between column with different superscript differed significantly (P<0.05)*

### Lesion score, oocyst index and OPG measured after coccidial challenge

Mean lesion score, oocyst index and OPG observed in *E. tenella* infection at d 4, 7 and 14 pi have been presented in table 2. The mean lesion score showed increasing trend till d 7 pi and then started decreasing upto d 14 pi. The highest mean lesion score was recorded on d 7 pi, while lowest was observed on d 14 pi. The birds challenged with dose 2 (20,000 sporulated oocysts) showed signs of thickened caecal walls, severe haemorrhages with bloody cores in the caeca and had resulted in higher lesion score at all the intervals. Oocyst index was maximum on d 7 pi and minimum on d 14 pi. The mean OPG count was also found to be highest on d 7 pi. On d 4 pi the OPG count was not observed in any of the Kadaknath birds. It was found to be highest on d 7 and declined on d 14 pi in both the treatment group. The mean lesion score and oocyst index value was maximum at d 7 pi and on third interval (d 14 pi), it declined to lowest. In the present study, observed OPG count, lesion score and oocyst index were lower as compared to the previous reports. Gabriel *et al.* (2003) reported significantly higher lesion score (+4) among infected treatment groups in Ross broiler chicken. Further, the oocyst outputs were significantly higher in infected birds exposed to the highest dosage. Pansare and Lonkar (2009) have also reported higher mean values for OPG and lesion score (+2.66 to +3.66) in WLH chickens challenged with 20,000 oocyst of *E. tenella* on

21st day of age. Likewise, Raman *et al*. (2011) reported +4 gross lesion score in broiler chicken at 3rd weeks of age, inoculated with $1 \times 10^2$ to $2 \times 10^3$ numbers of oocyst of *Eimeria* species. Similar results of higher lesion score (2.17 ± 0.21 to 3.08 ± 0.19) were also obtained by Mondal *et al.* (2011) in Cobb-100 broilers when inoculated with 10000 and 20000 sporulated oocysts of *E. tenella*.

**Table 2.** Mean (±SE) of lesion score, oocyst index and OPG at day 4, 7 and 14 post infection

| Interval | Groups | Lesion score | Oocyst index | OPG |
|---|---|---|---|---|
| Day 4 | Control | $0.00^{c} \pm 0.00$ | $0.00^{d} \pm 0.00$ | $0.00^{d} \pm 0.00$ |
| | T1 | $1.00^{ab} \pm 0.00$ | $1.00^{b} \pm 0.00$ | $0.00^{d} \pm 0.00$ |
| | T2 | $1.25^{ab} \pm 0.25$ | $1.00^{b} \pm 0.00$ | $0.00^{d} \pm 0.00$ |
| Day 7 | Control | $0.00^{b} \pm 0.00$ | $0.00^{c} \pm 0.00$ | $0.00^{d} \pm 0.00$ |
| | T1 | $1.50^{ab} \pm 0.29$ | $1.00^{b} \pm 0.25$ | $5282.0^{b} \pm 146.0$ |
| | T2 | $1.75^{a} \pm 0.25$ | $1.50^{a} \pm 0.29$ | $8981.0^{a} \pm 339.0$ |
| Day 14 | Control | $0.00^{c} \pm 0.00$ | $0.00^{d} \pm 0.00$ | $0.00^{d} \pm 0.00$ |
| | T1 | $0.75^{b} \pm 0.25$ | $0.50^{c} \pm 0.29$ | $377.0^{c} \pm 19.00$ |
| | T2 | $1.00^{ab} \pm 0.00$ | $0.75^{bc} \pm 0.25$ | $798.0^{c} \pm 42.00$ |

*Values within column (between rows) with different superscripts differed significantly ($p < 0.05$)*

No mortality was observed in any of the Kadaknath birds due to coccidial challenge in either of the treatment group over the entire study period. In contrary, Bumstead and Millard (1987) reported mortality among different inbred lines of chicken when inoculated with standard dose of 10,000 freshly sporulated oocysts of *E. tenella* and Shojaei (2014) also reported substantially higher mortality in Ross and Arbor Acres strains of broiler when challenged with dose of 50000 of sporulated oocysts of mixed *Eimeria* infection. The findings of present study revealed that the Kadaknath breed of chicken is less susceptible to present dose of coccidial infection. The reasons may be that the dosage of coccidial infection was not fatal enough to cause mortality in the challenged birds. The interaction of disease resistance genes i.e. MHC genes and non-MHC genes might have influenced the outcome of host response to coccidial infection.

On comparison with the findings of previous reports, Kadaknath breed showed comparatively less OPG count, lesion score and oocyst index during post coccidial infection. The oocyst index and lesion scoring are often considered as the most important indicators for coccidiosis evaluation, it can be realized that in the similar rearing conditions the economic losses due to coccidiosis can be substantially reduced by selecting Kadaknath birds in poultry rearing practices.

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