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Development of attenuated precocious lines of Eimeria tenella through chicken passage

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Abstract: *The field isolates of Eimeria tenella was attenuated by serial passage through chicken, of the first oocysts produced during infection. The selection pressure resulted in a reduction in the prepatent period of the parasite, shown to be due to the selection of a line predominantly with lesser number of generations of schizonts. The precocious lines had a reproductive potential much lower than that of the parent strain and it was significantly less pathogenic. These precocious lines could be useful as a vaccine inoculum to immunizing birds against chicken coccidiosis.*

Keywords: *Eimeria tenella, Precocious line, Vaccine, Chicken Passage*

I. Introduction

The apicomplexan parasite of chicken namely Eimeria can be attenuated by repeated passage in chicken embryos or by serial passage in chicken to produce "precocious lines". Precocious lines of Eimeria are populations that complete their endogenous life cycle in the host more quickly than a wild type parent strains. As repeated attenuation of such virulent isolates of Eimeria in natural host, resulted in early maturation of oocysts with a marked reduction in pathogenicity in the host intestinal tissue. The endogenous stages of the attenuated "Precocious" lines of Eimeria spp., may be depleted in numbers with reduction in size of generations (Jeffers, 1975; McDonald et al., 1982; Shirley, 1989).

Jeffers (1975) was the first to obtain an attenuated precocious line of Eimeria tenella (Wisconsin Strain) through serial passages in the chicken of the first oocysts produced during infection. This was characterized not only by an abbreviated life cycle but also by significant attenuation of virulence. The second generation meronts of precocious line were smaller and developed faster than those of the parent strain producing few or no second or third generation meront (McDonald et al., 1986a). Attenuation of virulence was found to increase with continuing selection and was continued until each line induced no, or minimal, deleterious effects on the body weight gains of infected birds. Despite a reduced multiplication within the intestine, precocious lines were able to stimulate a protective immunity virtually as good as that induced by their pathogenic parents. Recently, many live multivalent vaccines containing one (Eimeria vax)) or more species (Paracox, Livacox) of precocious line of Eimeria was developed and successfully used in field conditions (Shirley and Bedrnik, 1997).

In numerous occasions, failures using exotic vaccines due to strain variation have been observed. This paper describes the development of precocious line of Eimeria tenella field isolate first of its kind using local field isolates from India through chicken passage as a part of DBT funded project on Development of multivalent oral pellet vaccine against chicken coccidiosis in India.

II. Materials and Methods

Source of Eimeria tenella

The collected field isolates of Eimeria tenella parasites from different parts of Southern India were previously confirmed and characterized by Saira Banu eta al ., (2009) were selected based on its virulence and pathogenicity.

Development of Pure line of Eimeria tenella

The sporulated oocysts of the field isolates were serially diluted from the starting of $1X10^3$ oocysts to single oocysts. Thus prepared single oocysts were poured on the slide along with the melted agarose. The agarose gel containing single sporulated oocysts was selected under the microscope and the gel slice containing single oocysts was used to infect the birds for the pure line development.

Derivation of Eimeria tenella precocious line

The precocious line of Eimeria tenella was obtained by selecting for early development of oocysts (Jeffers, 1975). The first passage oocysts were collected after 122hrs post inoculation and the selection pressure was alternatively applied and relaxed (Eckert et al. 1995).

Pathogenicity and Immunogenicity studies

Fifteen day old Rossn 308 chickens were maintained in a group of 20 birds and were given $2X10^4$ numbers of precocious line of Eimeria tenella. Oocyst output and the lesion scores of infected groups were compared on day 6 with the uninfected control groups.

Calculation of OPG and Lesion Scoring

The oocyst per gram of feces was assessed using three chambers McMaster egg counting Chamber (Haug et al., 2007). The lesion scores were derived based on Raman et al., (2011).

III. Results and Discussion

For the first two passages with the selection for early development, oocysts were isolated from the feces at 122 hours post infection. With continued selection oocysts were collected at increasingly earlier times and by 15^{th} passage oocysts were collected at 96 hours of post infection. With subsequent selections till 20 passages were able to produce the oocysts at 72 hours of post infection. Hence, the prepatent period of the selected line of Eimeria tenella over 20 passages reduced from 122hrs to 72 hours (Fig-1). This selection pressure can result in the elimination of one or more of the lateral developing asexual stages (Merons).

The pathogenicity by means of lesion score was reduced from +4 to +1 by the end of 20 passages when the birds inoculated with the same number of oocysts ($2X10^4$) (Fig 2). Oocysts were found 48 hours earlier in the feces from chickens inoculated with the precocious strain of Eimeria tenella. The peak oocyst production was observed on 7^{th} day in the parent strain, and it was reduced to 4days in the case of precocious strain. Total oocyst production was reduced to 50%, ie after 20 passages the total yield of oocyst output was 50% reduced than the parent strain (Fig 3). Considering overall performance of birds, precocious line 72hrs was less pathogenic with the minimal oocyst output of $8X10^3$ oocysts.

The potential use of precocious lines of Eimeria species as potential use for bird immunization is well documented (Shirley et al., 1995; Vermeulen et al., 2001). In the development of an effective safe live vaccine for coccidiosis, the first priority is to produce attenuated lines of Eimeria. In earlier studies, attenuation of E.tenella and E.mitis was achieved by serial passage in the chorio- allantoic membranes of developing chicken embryos (Long, 1972). These strains had lowered pathogenicity compared with the parent lines from which they were derived but not retained marked immunogenicity. Even though there are many available precocious vaccine (Paracox, Livacox), and good report on protection those laboratory strains failed to give protection in the field condition s due to strain variations. In this experiment, the virulent field eimeria tenella isolates were successfully isolated from chicken feces and selected for precocity by passage through chickens. In conclusion, it is suggested that the attenuated precocious line of Eimeria tenella local isolate, having retained sufficient productiveness to immunize chickens in litter is probably more suitable than the exotic vaccines when administered along with the E. acervulina and E. maxima can be used as a better vaccine innocula for the development of multivalent vaccine against chicken coccidiosis.

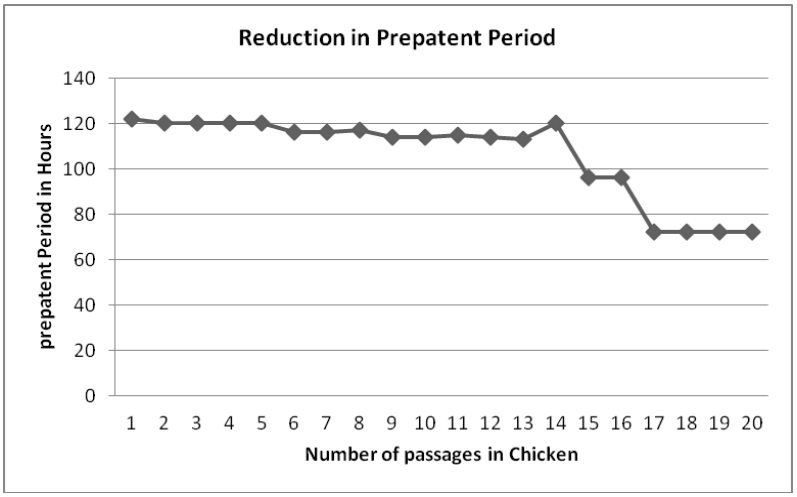

Fig 1: Reduction in Prepatent period in Precocious line

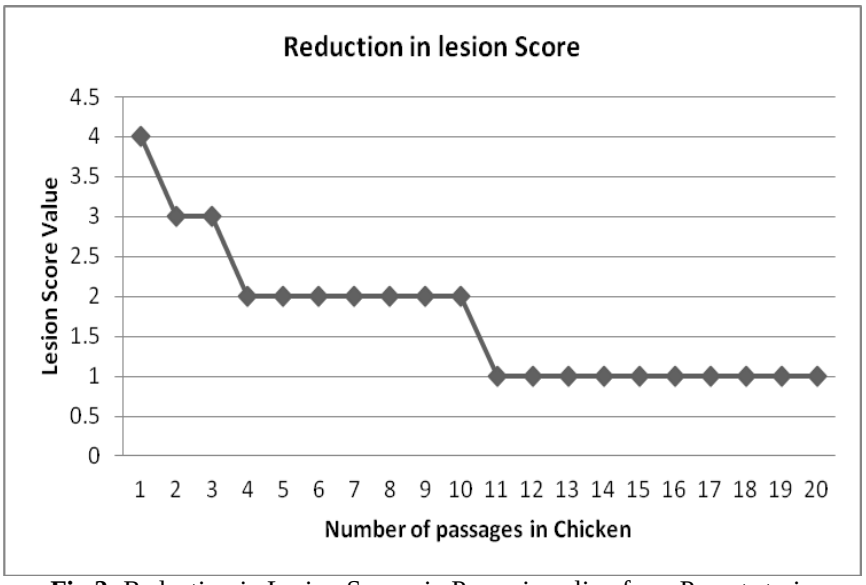

Fig 2: Reduction in Lesion Scores in Precocious line from Parent strain

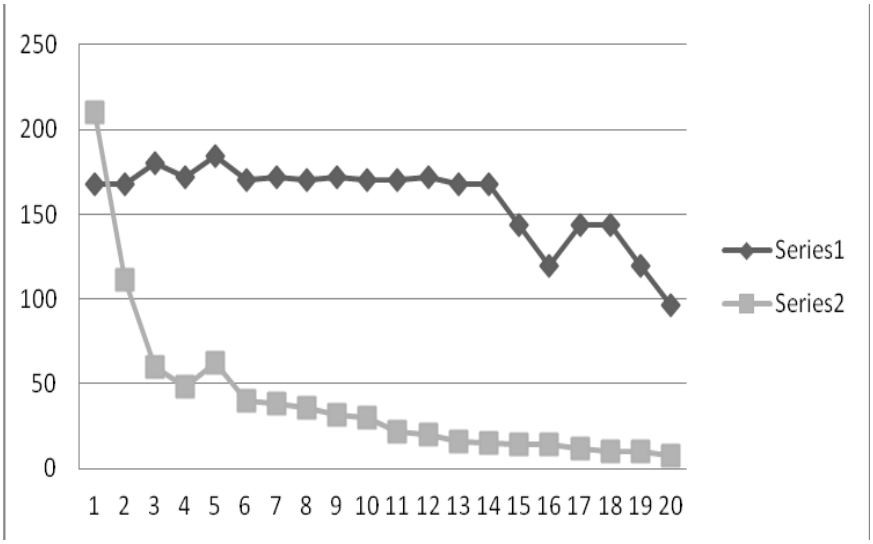

Fig 3: Comparision of oocyst output with relavence to the peak oocyst production in hours

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