DEVELOPMENT OF A PRECOCIOUS STRAIN OF *Eimeria tenella*

By

SANGEETHA. R.

THESIS

Submitted to the Kerala Agricultural University
in partial fulfilment of the requirements
for the degree of

Master of Veterinary Science
in
Veterinary Parasitology

COLLEGE OF VETERINARY AND ANIMAL SCIENCES
KERALA AGRICULTURAL UNIVERSITY
MANNUTHY, THRISSUR - 680651
KERALA, INDIA

2001

DECLARATION

I hereby declare that the thesis entitled **"DEVELOPMENT OF A PRECOCIOUS STRAIN OF *Eimeria tenella*"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy
29.08.01

R. Sangeetha

SANGEETHA. R.

CERTIFICATE

Certified that the thesis entitled **"DEVELOPMENT OF A PRECOCIOUS STRAIN OF *Eimeria tenella*"** is a record of research work done independently by **Sangeetha, R.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

Dr. K. Devada
Assistant Professor,
Department of Veterinary Parasitology,
College of Veterinary and Animal Sciences,
Mannuthy.

Mannuthy
29.01.01

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Sangeetha, R.,** a candidate for the degree of **Master of Veterinary Science** in **Veterinary Parasitology**, agree that the thesis entitled **"DEVELOPMENT OF A PRECOCIOUS STRAIN OF *Eimeria tenella*"** may be submitted by Sangeetha, R. in partial fulfilment of the requirement for the degree.

29.08.01

Dr. K. Devada
Assistant Professor
(Chairperson, Advisory Committee)
Department of Veterinary Parasitology
College of Veterinary and Animal Sciences,
Kerala Agricultural University,
Mannuthy.

Dr. H Subramanian,
Associate Professor and Head,
Department of Veterinary Parasitology
(Member)

Dr. Amritha Viswanath
Associate Professor,
Department of Poultry Science
(Member)

Dr. C.R. Lalitha
Associate Professor,
Centre of Excellence in Veterinary Pathology
(Member)

13/11/01
EXTERNAL EXAMINER
Dr. D. Kumar
Professor & Head
Dept. of Parasitology
Rajiv Gandhi College
of Vety. & Anl Sc
Pondicherry-9

ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude to ***Dr.K.Devada****, Assistant Professor, Department of Veterinary Parasitology and chairperson of the advisory committee for her valuable guidance, helpful suggestions, constant encouragement and motherly affection extended throughout this work.*

I am greatly indebted to ***Dr.H.Subramanian****, Associate Professor and Head in-charge, Department of Veterinary Parasitology,* ***Dr.Amritha Viswanath****, Associate Professor, Department of Poultry Science,* ***Dr.C.R.Lalitha****, Associate Professor, Centre of Excellence in Pathology, for their interest, help and co-operation as members of the advisory committee.*

I am grateful to Dr.S.Sulochana, Dean (Retd.) and Dr.K.N.Muralidharan Nair, Dean, College of Veterinary and Animal Sciences, Mannuthy for providing the facilities needed for this work.

I express my sincere gratitude to Dr.Lucy Sabu, Assistant Professor, Department of Veterinary Parasitology for her valuable guidance and friendly support.

Special thanks are due to Dr.Abdul Basith, Professor, Department of Parasitology, Madras Veterinary College, Chennai for his advice and guidance with regard to single oocyst isolation technique.

The facilities provided by Dr.K.V.Valsala, Professor and Head, Centre of Excellence in Pathology, for histopathology is greatly acknowledged.

I thank Smt.U.Narayanikutty, Assistant Professor and Head in-charge, Department of Statistics and Smt.K.P.Santha Bai, Senior Programmer for their help in analysis of data.

I remember with gratitude the help rendered by Dr.Leo Joseph and Dr.Narayanankutty and other staff members of the Department of Poultry Science for providing the chicks and chick feed for the experiments.

My heartfelt thanks goes to Dr.S.Suraj, M.V.Sc. scholar, Centre of Excellence in Pathology and Dr.C.V.Sreeranjitkumar, Research Associate, Department of Microbiology for their kind and patient help in taking photomicrographs.

I also thank all the non-teaching staff of the Department of Parasitology for their kind co-operation.

I express my deepest gratitude to my seniors Dr.N.Bhaskar and Dr.A.Kandasamy for their friendly encouragement. With great pleasure, I thank Dr.Sreevidya Nambiar for her help, sisterly affection and concern throughout the work. I also thank Dr.Shameem for the help rendered during various stages of my work.

I offer my thanks to Dr.Bindiya Liz Abraham, Dr.Geetha, Dr.P.M.Priya, Dr.Princy Thomas, Dr.Rony Ray John, Dr.P.R.Sindhu and Dr.Jayshree for their warm friendship and support.

I am greatly indebted to Dr.R.Lakshmi, Dr.S.Rajalakshmi, Dr.Lydia Priscilla, Dr.A.Sabiha Mahaboob Khadari and Dr.Binsy Mathew for their warm love, friendship, help and moral support without which this task could not have been accomplished.

I also thank Dr. Vijayakumar, Dr. Rajkumar and Dr. S. Kantharaj for their friendship and timely help.

With immense pleasure, I would like to acknowledge with deepest gratitude the moral support, love and blessings extended by my parents, brother, aunty Miss.Girija and other relatives.

I am greatly thankful to M/s.J.M.J. Computer Centre, Thottapady for the neat typing of the thesis.

I thank the KAU for the grant of fellowship.

Above all, I thank the Almighty for his blessings, which has helped me to complete this task, successfully.

R.Sangeetha

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INTRODUCTION

1. INTRODUCTION

Coccidian parasites have long drawn the attention of a very large number of protozoologists and veterinarians as causative agents of the dreadful disease, 'coccidiosis'.

Coccidial infections of chicken are ubiquitous and form one of the most frequently occurring poultry diseases (Biggs, 1982). Infected chicks showed listlessness, anorexia, weight loss and haemorrhagic diarrhoea and subsequent death. Disease could be diagnosed from the clinical signs, faecal examination for oocysts and observation of intestinal lesions at autopsy (Hofstad, 1978). Gigi George (1997) recorded the prevalence of coccidiosis as 21.13 per cent by examination of 355 viscera of birds brought for postmortem at the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, during 1996 - 97.

The current importance of coccidiosis to the world poultry industry is reflected by a recent estimate that losses due to this disease total to $ 800 million per annum (Williams, 1994). Furthermore there is an increased

concern about the rising levels of drug resistance, failure in the discovery of new chemical entities, high costs for the development of new anticoccidial drugs and consumer pressure to phase out the inclusion of additives in the diet of food animals.

As a result, in recent years, there has been a refocusing of research on the biological means of control rather than chemical control with regard to coccidiosis. On the contrary, it is seen that immunological control is the only major practical alternative to chemotherapy.

Although virulent live vaccines against chicken coccidiosis have been available commercially for some years, their safety is doubtful as birds are not uniformly exposed to the infection with some remaining resistant and others developing clinical coccidiosis.

Jeffers (1975) showed that repeated selection for the initial oocysts produced during a coccidial infection yielded parasites with an abbreviated life cycle and a concomitant reduction in pathogenicity. Such selected parasites were referred to as **precocious strains** and form a suitable material for inclusion in live vaccines.

Many precocious strains, representative of both avian and mammalian species of *Eimeria* have been selected and used in immunisation trials worldwide (Shirley, 1993).

The utility of the concept of selection for precocious development as a basis for production of live attenuated vaccines for the control of coccidiosis is receiving increased attention. Considering the advantages and merits of precocious strain vaccines over other means of control, the present study was taken up with the following objectives:

1. to develop a precocious strain of *Eimeria tenella*, the causative organism of caecal coccidiosis in chicken and

2. to compare the oocyst morphology, oocyst production potential and the clinical changes produced by the stabilised strains and precocious strains in chicken.

2. REVIEW OF LITERATURE

Coccidiosis is one of the most prevalent diseases confronting the poultry industry. Nine species of coccidia are responsible for this disease in poultry among which *Eimeria tenella* is the most pathogenic and more frequently occurring species.

2.1 Prevalence of *Eimeria tenella*

A survey of coccidiosis conducted by Jagadish Babu *et al.* (1976) in poultry at Bangalore, revealed that, out of 1043 autopsies, 274 cases showed lesions of coccidiosis, of which 81 cases (28.9 per cent) were due to *E. tenella*.

Srinivasa *et al.* (1989) conducted postmortem of 4603 chicken in Kurnool district, Andhra Pradesh, between 1983 and 1988 and reported that caecal and intestinal coccidiosis accounted for 11.6 per cent mortality.

Coccidiosis was found to be more prevalent during cold months of the year by Jahurulkarim *et al.* (1994).

Rana and Tikaram (1994) stated that *E. tenella* was the most pathogenic and predominant species prevalent in semi arid parts of Hisar and Jind districts of Haryana

and the mortality rates were 3.72 per cent and 6.34 per cent, respectively. Prevalence was high during summer.

In a prevalence study carried out by Panda *et al.* (1996) in Bhubaneswar, Orissa, six species were recorded of which *E. tenella* showed the highest prevalence rate of 65.38 per cent. Mortality due to coccidiosis was 11.75 per cent and of these, caecal coccidiosis accounted for 7.03 per cent. High mortality rate was recorded in the months of June and July and low in January.

Gigi George (1997) studied the prevalence of coccidiosis in broiler chicken. Out of 355 birds brought for postmortem at the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, from 1996 to 1997, mixed infections of *Eimeria* were found in 75 cases (21.13 per cent) of which *E. tenella* accounted to 72 per cent.

2.2 Confirmation of species

Morehouse and McGuire (1958) identified various species of *Eimeria* through measurement of oocysts, observation on the length of prepatent period and appearance of lesions.

Joyner and Long (1974) stated that a single criterion was usually insufficient for identification of Eimerian species. Details on the morphology of oocysts and other developmental stages of the parasites, their location in the hosts and characteristics of lesions produced, the timing of patent and prepatent period, host and site specificity and immunological specificity could be used as aids for species identification. According to them, the site specificity was a useful diagnostic guide in natural infections, especially where endogenous stages could be recognized such as the large second generation schizonts of *E. tenella*.

Jagadish Babu *et al.* (1976) identified the species based on morphological characters of oocysts and pattern of distribution of lesions in different segments of the intestines, grossly and microscopically.

Soulsby (1982) reviewed on the gross lesions, sporulation time, oocyst morphology, micrometry and prepatent period of various species of coccidia. These characters were specific to each species.

Long and Joyner (1984) stated that the distinctions between Eimerian species, could be made

based on morphological data, host and site specificity, pathogenicity, immunological specificity, prepatent period, sporulation time and enzyme variation.

Kucera (1990) identified different species of coccidia by microscopic examination of the stages in different regions of infected gut, measurement of oocysts and electrophoresis of lactate dehydrogenase and glucose phosphate isomerase.

Karim and Begum (1995) and Thakuri and Rai (1996) differentiated the species of *Eimeria* based on oocyst dimension, prepatent period and site of infection.

2.3 Vaccination trials based on precocious lines

Johnson *et al.* (1978) tried practical immunisation of chicks against coccidiosis using two precocious strains (Wis. F 96 and Wis F 125) and a strain used in commercial planned immunisation programme (coccivac). Flock immunity was equivalent between the control strain and higher doses of Wis F 125, but strain Wis F 96 did not adequately immunise the chicks.

Williams (1993) studied the efficacy of Paracox, a live attenuated vaccine comprising a stabilised suspension of precocious lines of seven species of

Eimeria. Results indicated that under commercial conditions this vaccine allowed satisfactory rearing of chicken without the use of prophylactic anticoccidial medication.

Eckert (1994) reviewed the progress made in vaccine development against coccidiosis. Two commercial vaccines based on precocious lines have been reported. Paracox (Pitman Moore Europe Ltd.) registered in several European countries could be applied via drinking water to chicken five to nine days of age. Results in over 20 million birds showed that it could give excellent protection against coccidiosis. Livacox (Biopharm) developed in Czech Republic also showed good vaccine efficacy and tolerance when given as a single dose via drinking water to seven to ten day old chicks.

Lightowlers (1994) reviewed the different vaccines used against coccidiosis in chicken and stated that substantial success has been achieved by the use of precocious line based vaccines.

Williams (1994) demonstrated the safety of Paracox, administered at the recommended dose when chicks were seven days old. The vaccinated birds performed

better than the unvaccinated controls, with no post vaccinal reduction in their weight gain. They also showed 10.4 per cent greater finishing weight, 7.2 per cent lower feed conversion ratio and 44.4 per cent lower mortality than the unvaccinated controls.

Shirley *et al.* (1995) conducted vaccination trials in broiler breeders and replacement layer flocks for control of avian coccidiosis using a live attenuated vaccine containing lines of seven species of *Eimeria* selected for precociousness. The vaccine was given in drinking water to 1,16,600 chicks. The occurrence of coccidial oocysts in the litter, postmortem lesions and clinical signs were markedly lower in the vaccinated birds than in the controls.

Rai *et al.* (1996) developed precocious lines of *E. tenella* and *E. acervulina* and used them for oral immunisation of five to six day old chicks at the rate of 10×10^3 sporulated mixed oocysts. A challenge infection was given with 20×10^3 oocysts on the 14^{th} day. The oocysts per gram (OPG) of faeces was significantly low in the case of immunised chicks (2×10^2 to 10×10^2) compared to the non-immunised chicks (2.5×10^3 to $10 \times$

10^3). None of the immunised chicks showed any clinical sign.

Williams *et al.* (2000) administered Paracox - an anticoccidial vaccine to a seven day old flock of commercial broiler breeder stock, in various management programmes and challenged with *E. necatrix*. The results confirmed the hypothesis that vaccinated birds kept on litter produced significant number of oocysts initially but produced hardly any oocyst upon a second homologous vaccination.

2.4 Recommended dose of *Eimeria tenella* oocysts

Jankiewicz and Schofield (1934) stated that doses of *E. tenella* oocysts upto 1.5×10^2 produced no symptom, while doses of 1.5×10^2 to 5×10^2 caused haemorrhage without any mortality in chicks. Heavy haemorrhage was produced by doses of 1×10^3 to 3×10^3 oocysts but only light mortality resulted. Doses of 3×10^3 to 5×10^3 oocysts produced marked haemorrhage and moderate mortality and doses of more than 5×10^3 oocysts caused heavy mortality.

According to Long *et al.* (1976) a dose of 1×10^2 to 5×10^2 *E. tenella* oocysts were needed for oocyst

output studies and that of 5 x 10^4 to 2 x 10^5 oocysts to produce pathogenic effects.

Kawaguchi *et al.* (1988) used 5 x 10^3 oocysts of *E. tenella* to develop a precocious line.

Rai *et al.* (1996) administered 1 x 10^4 sporulated *E. tenella* oocysts to five day old chicks to develop a precocious strain.

Rao *et al.* (1999) developed a precocious strain of *E. tenella* by passaging 5 x 10^3 oocysts per bird.

2.5 Single oocyst isolation

Long (1959) isolated a single oocyst of *E. maxima* by micromanipulation using a pipette with an internal bore of 30 µm. The oocyst was discharged on to a cellophane square on a microscopic slide and after confirmation that no other oocysts were present, the cellophane square was transferred to a No.4 gelatin capsule which was then introduced into the crop of the experimental chicken.

Edgar and Siebold (1964) outlined a method of obtaining pure line cultures by single oocyst isolation. Oocysts, as single were given separately to one to five

day old coccidia free chicks which were killed on the seventh day pi. The oocyst suspension after sporulation was passed blindly through another batch of coccidia free chicks. This was continued through different groups of chicks and the resultant oocysts furnished the pure line culture.

In the absence of a suitable micromanipulator, Long *et al.* (1976) showed that isolated oocysts could be picked out manually under the microscope using a short Pasteur pipette drawn out to a fine tip. It was noted that 30 to 50 per cent of the single oocyst infected birds developed patent infections.

Karim and Trees (1990) used the technique described by Long (1959) to derive monospecific strains of *E. tenella, E. acervulina, E. maxima, E. brunetti* and *E. necatrix* by infecting seven day old chicks with a single oocyst each.

Basith (2000) used agar pieces coated on glass slides for satisfactory results. The drop containing oocysts was placed on a glass slide and was serially diluted microscopically till the number of oocysts in the drop was reduced to one. This drop containing a single

oocyst was placed on an agar piece and after confirmation that no other oocyst was present, this agar piece was introduced directly into the oesophagus of experimental chick.

2.6 Development of stabilised strain

Jeffers (1975) derived the stabilised strain Wis-C from the Wisconsin strain of *E. tenella* by orally administering oocysts to coccidia free chicks, and harvesting the oocysts from the caecal contents at 168 hr pi.

Similarly, Rao *et al.* (1999) harvested the caecal contents after sacrificing the *E. tenella* infected birds at 168 hr pi. The sporulated oocysts thus harvested were used in subsequent passages and at the end of 10 cyclical passages, they were designated as the stabilised strain of *E. tenella*.

2.7 Development of precocious strain

Jeffers (1975) derived a precocious strain of *E. tenella* (Wis F) from Wisconsin strain (Wis) by orally administering oocysts to coccidia free chicks and harvesting the oocysts from the caecal contents at 125 hr pi. He noted a substantial attenuation of the parasites

only after 10 generations of selection for early development.

McDonald and Shirley (1984) compared the endogenous developmental stages of a precocious line (HP) and its parent Houghtan (H) strain of *E. mitis*. The period of life cycle of the precocious line was shorter than that of the parent strain. Gametocytes appeared to develop from third generation as well as from fourth generation merozoites. Also sporozoites of the precocious line transformed to trophozoites before those of the parent strain. The differences between the life cycles of the two parasites accounted for the lower reproductive potential of the precocious line.

Shirley and Bellatti (1984) derived a precocious line of *E. necatrix* after 17 generations of selection, thereby reducing the prepatent period to 117 hr from 138 hr. There was a marked attenuation of virulence despite which, the precocious line retained much of its immunogenicity. The precocious line appeared more stable than the egg adapted line, which reverted to virulence and was markedly less immunogenic than its parent strain.

Shirley *et al.* (1984) selected a precocious line of *E. praecox* and studied its characteristics. The prepatent time was found to be reduced from 84 to less than 64 hr. Endogenous study revealed the depletion of the fourth generation schizonts. The multiplication rate was 10 per cent less than that of its parent strain. The precocious line obtained was less pathogenic and chicken given small number of oocysts were almost completely immune to challenge with either the parent or any of the field strains.

Joyce *et al.* (1986) compared the pathogenicity, immunogenicity and endogenous development of a precocious line of *E. brunetti* with its parent strain. The prepatent period of the precocious strain was observed as 75 hr and that of the parent strain as 120 hr. The precocious line was less pathogenic than the parent, caused less weight depression and lower lesion scores in chicken and was highly immunogenic protecting against a challenge of parent strain which caused 83 per cent weight depression and 13 per cent mortality in non-immunised challenged controls. The third generation schizonts were almost completely eliminated in the precocious line with regard to the endogenous development.

McDonald *et al.* (1986) developed a precocious line of *E. maxima* which showed a reduction in the prepatent period from 120 to 107 hr. Endogenous studies disclosed that the number of schizogonies of the precocious lines were reduced due to the earlier onset of gametogony.

Kawaguchi *et al.* (1988) used the parent NIAH strain of *E. tenella* to produce a precocious line (NIAH/P20). Selection was carried on for 20 generations and the prepatent period was reduced to 112 hr, about 24 hr shorter than the parent NIAH strain. The precocious line was significantly less pathogenic, but it maintained the level of reproductive potential preceding the selection.

Hu-Jing Hui *et al.* (1998) studied the immunogenicity and virulence of *E. brunetti* precocious strain and concluded that this strain could be used for the development of attenuated coccidia vaccines.

Liu-Qun *et al.* (1998) compared the reproduction and immunogenicity of the three lines of *E. tenella viz.* precocious line, chick embryo adapted line and the virulent line. The prepatent period and the oocyst

production were the least with the precocious line developed.

Montes *et al.* (1998) developed a Spanish precocious strain of *E. necatrix* in chicks of two to six days of age. Oocysts were collected from the caecal contents of chicks sacrificed at a predetermined time after infection. After 20 passages, the prepatent period was reduced by 30 hr (from 148 to 118 hr). The pathogenicity was substantially reduced when compared to the parent strain.

Hu-Jing Hui *et al.* (1999) attenuated *E. brunetti* by repeated passages in chicken and found that the precocious strain had a prepatent period of 108 hr which was 12 hr shorter than the original strain. The pathogenicity was also significantly reduced.

Rao *et al.* (1999) developed a precocious strain of *E. tenella* by isolating oocysts which appeared early in the faeces during serial passages of the parasites in two to three week old White Leghorn chicks. Oocysts were first collected directly from the caeca after sacrificing the birds at 128 hr pi. The sporulated oocysts were then subsequently passaged applying selection pressure in

increments of one hr reduction per passage. Oocysts were collected during about six hr after the first finding of oocysts in the faeces and sporulated oocysts were subsequently repassaged to chicks at a dose of 5 x 10^3 oocysts per bird through 15 generations.

2.8 Comparison of precocious and stabilised strain

2.8.1 Prepatent period

Jeffers (1975) achieved a reduction of 12 hr in the prepatent period of the precocious strain of *E. tenella*.

Kawaguchi *et al.* (1988) produced a precocious strain of *E. tenella* with a prepatent period of 112 hr, which was about 24 hr shorter than that of the control strain.

Rao *et al.* (1999) obtained a prepatent period of 120 hr in the precocious strain of *E. tenella* which was 18 hr less than that of the stabilised strain.

2.8.2 Oocyst morphology

Jeffers (1975) opined that the increase in the developmental rate of strain Wis F was accompanied by a reduction in the oocyst size. At 168 hr pi, Wis C oocysts had a length of 21.5±0.19, width of 17.4±0.10 and a shape

index (L/W) of 1.23. At 115 hr pi Wis F showed a length of 17.8±0.14, width of 14.7±0.10 and shape index of 1.21. But there was no significant difference in the mean oocyst dimension when oocysts were harvested at 168 hr pi which suggested that the reduction in oocyst dimensions of strain Wis F was not a direct result of selection for precociousness but rather a function of time permitted for growth of macrogametes.

Rao *et al.* (1999) did not find any significant difference in the oocyst morphometry of *E. tenella* wild and precocious strains. The mean oocyst dimensions were 21.9 x 16.4 µm (19 to 24 x 16 to 23 µm) and 20.7 x 15.8 µm (20 to 22 x 14 to 20 µm) for the wild and precocious strains, respectively. The shape index of the precocious strain was 1.31 while that of the wild strain was 1.33.

2.8.3 Oocyst production potential

Jeffers (1975) recorded the comparative rate of oocyst production of strain Wis C and strain Wis F of *E. tenella* after nine generations of selection for precociousness. It was found that at earlier hours, the precocious strain produced more oocysts than the stabilised. The oocyst production was 24 x 10^4 at 108 to 120 hr and 178 x 10^4 at 120 to 132 hr pi for the

precocious and zero and 83 x 10^4 during the same time for the stabilised. During the later hours, at 160 to 180 hr pi the stabilised strain produced more oocysts, the number being 525 x 10^4 while the precocious strain produced 144 x 10^4 oocysts.

Kawaguchi *et al.* (1988) did not detect any remarkable difference between the precocious and the parent lines of *E. tenella* in the number of oocysts excreted throughout the experimental period.

Shirley (1993) reported that precocious line of *E.maxima* took 107 hr and produced only 70 x 10^3 oocysts from an initial dose of 100 oocysts in comparison to 30 million oocysts produced by the virulent strain.

Liu Qun *et al.* (1998) observed that the oocyst production of the precocious line of *E. tenella* was 24.75 per cent less than that of the virulent strain.

According to Rao *et al.* (1999) the oocyst production potential assessed by oocysts per gram (OPG) of faeces of the precocious strain was comparatively low when compared to the unselected parent strain. At 120 hr pi, the OPG was higher (1.5 x 10^3) for the precocious strain than that of the wild strain (7 x 10^2). The OPG

counts of the precocious strain and control strain at 128, 136, 144, 160 and 168 hr pi were 1.15 x 10^3, 9 x 10^3, 6.3 x 10^3, 2.5 x 10^3 and 4.5 x 10^3 and 1.65 x 10^3, 9.2 x 10^3, 6.1 x 10^3, 5 x 10^3 and 8 x 10^3, respectively.

2.8.4 Clinical symptoms and gross changes of caeca at autopsy

Johnson and Reid (1970) devised a zero to +4 lesion scoring system for *E. tenella*. The lesions were scored as follows:

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. Caecal 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.

Jeffers (1975) showed that infective doses of upto 5 x 10^5 oocysts per bird of the precocious strain (Wis F) of *E. tenella* were practically innocuous. Surprisingly the birds exhibited only mild clinical signs of infection. The caecal lesion score of the precocious strain was significantly less than the control.

Kawaguchi *et al.* (1988) noted comparatively less clinical signs and caecal lesions in the precocious strain than in the control.

Rao *et al.* (1999) reported that the appearance of blood in the faecal droppings was marked in the wild strain from 128 to 136 hr pi while little or no blood was seen in the faeces of chicks infected with the precocious strain of *E. tenella*. The average lesion scores of the wild and precocious strains were 3 and 2, respectively, where, 1 - microscopic evidence of coccidia, 2 - light gross lesions, 3 - moderate gross lesions and 4 - maximal gross lesions.

2.8.5 Body weight

At a dose level of 5 x 10^5 oocysts per bird, Jeffers (1975) found that the average survivor weight gain (g) in White Leghorn chicks of 24 days of age, with

respect to Wis, Wis C and Wis F strains were 38.9±7.1, 20.7±8.2 and 86.1±2.3, respectively.

Kawaguchi *et al.* (1988) found that the adverse effects of the parasite infection on body weight gain were relatively greater in NIAH strain than in NIAH/P20 line.The relative body weight gain percentage in 18 day old birds infected with $5x10^4$ oocysts of NIAH/P20 and NIAH lines per bird were 81.5 and 46.3, respectively.

In the experiments of Rao *et al.* (1999) the precocious strain inoculated chicks showed a marginal increase of 2.14 per cent whereas chicks inoculated with wild strain of *E. tenella* recorded only 1.57 per cent body weight gain on the eighth day pi.

2.8.6 Mortality

Jeffers (1975) found that at a dose level of 5 x 10^5 oocysts per chick, four out of ten chicks died when given Wis C strain while there was no mortality with the precocious strain.

In the experiments of Kawaguchi *et al.* (1988) the rate of mortality was found similar in both groups infected with precocious and parent strains.

Rao *et al.* (1999) also found no significant difference between the groups in terms of mortality. Five out of ten chicks died due to inoculation with precocious strain, while seven out of ten died due to inoculation with a wild strain of *E. tenella*.

2.8.7 Histopathology

Jeffers (1975) examined the histological sections of parasitised caecal tissues and stated that the lack of pathogenicity due to Wis F strain was as a result of defective second generation schizogony. He noticed the mature second generation schizonts of the attenuated strain (Wis F) to be smaller and they contained a few number of merozoites than those of the virulent strain (Wis C).

Histological observations carried out by Kawaguchi *et al.* (1988) revealed significant differences between NIAH and NIAH/P20 lines in the rate of development, size of the mature second generation schizonts and topographical distribution of the schizonts in the lamina propria of the caecal villi.

In the NIAH infected chicks, mature first generation schizonts were observed at 55 hr pi and mature

second generation schizonts at 96 and 103 hr pi in the lamina propria and near the muscular layer of caeca. Oocysts appeared at 144 hr pi.

In the NIAH/P20 line infection, mature first generation schizonts appeared as early as 48 hr pi while the mature second generation schizonts which were obviously smaller than those of NIAH strain were found at 72 and 79 hr pi in the more superficial parts of the lamina propria.

Many gametocytes were found at 120 hr pi which was about 24 hr earlier than their appearance with the parent NIAH infection. Oocysts appeared as early as 120 hr pi in the caecal sections.

The results of the endogenous studies by Rao *et al.* (1999) indicated accelerated growth of the asexual developmental stages. Mature first generation schizonts of the precocious strain appeared as early as 48 hr and many gametocytes were found 120 hr pi which was approximately 24 hr earlier than the unselected parent strain.

3. MATERIALS AND METHODS

3.1 Prevalence of *Eimeria tenella*

Postmortem registers maintained at the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy were referred to for understanding the extent of *Eimeria tenella* infections and consequent mortalities in birds during the period from June 2000 to May 2001.

3.2 Isolation of *Eimeria tenella* from a field outbreak

Caeca were collected from a typical field case of caecal coccidiosis during postmortem of a bird at the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy. Separation of oocysts from the caecal contents was done by a combination of sedimentation and floatation technique (Long *et al.*, 1976). The samples were mixed with water, filtered and concentrated by gravity sedimentation. The supernatant fluid was removed and the sediment was agitated after mixing with saturated sugar solution and subjected to centrifugal floatation technique.

The oocysts were then recovered with a Pasteur pipette from the surface and placed in water in a small

beaker. This fluid was then centrifuged to remove surplus water and excess sugar solution and the oocysts were finally suspended in shallow layers of two per cent potassium dichromate solution at room temperature for sporulation.

3.3 Confirmation of *Eimeria tenella*

The oocysts were confirmed to be that of *E. tenella* by observing the sporulation time, oocyst morphology, micrometry and the prepatent period in experimental infection.

3.3.1 Sporulation time

The oocysts were suspended in shallow layers of two per cent potassium dichromate solution and kept at room temperature. Drying was avoided by regularly adding potassium dichromate solution. The mixture was aerated at regular intervals to aid sporulation. The sample was examined at short intervals under the microscope to observe the process of sporulation. The sporulation time for the species was recorded when 50 per cent of the oocysts in the sample had undergone sporulation.

3.3.2 Oocyst morphology and micrometry

The morphological details of both sporulated and non-sporulated oocysts were studied microscopically. The

size of the oocysts were measured by micrometry. Measurements of atleast 100 oocysts were taken and the average determined.

3.3.3 Prepatent period

Six, day-old White Leghorn male chicks were purchased from the University Poultry Farm, Kerala Agricultural University, Mannuthy and were reared under hygienic conditions, following the standard managemental practices. Standard chickmash without any anticoccidials and clean water were provided *adlib.* Sporulated oocysts of *E. tenella* at the rate of 5 x 10^2 oocysts per bird were given orally to determine the prepatent period.

The time interval between the inoculation of oocysts and recovery of oocysts from the faeces was noted. This trial was repeated three times for more accurate results.

3.4 Single oocyst isolation technique

The technique described by Basith (2000) was followed. Sporulated oocysts of the already confirmed species of *E. tenella* were washed repeatedly to remove excess of potassium dichromate. They were then suspended in distilled water. The oocysts were drawn by means of a

fine Pasteur pipette and placed on a glass slide. The drop containing oocysts was serially diluted on the glass slide microscopically till the number of oocysts in the drop was reduced to one. Agar solution was then poured onto another glass slide and cut into several pieces of 0.2 sq. cm size, after solidification. The drop containing a single oocyst was then picked up with a fine Pasteur pipette and placed on one of the agar pieces. It was examined under the microscope to confirm the absence of other oocysts along with it.

3.5 Experimental infection

The agar piece was carefully taken and introduced directly into the oesophagus of twelve, three day old White Leghorn chicken raised separately under coccidia free conditions. Droppings of these chicken were examined for four to nine days pi for establishment of the infection.

Three uninfected birds were reared as controls under the same conditions.

3.6 Propagation of pure line of *Eimeria tenella*

The oocysts obtained by single oocyst isolation technique were collected from the droppings and caeca of

the infected birds between 146 and 168 hr pi. After sporulation, the oocysts obtained from each bird were amplified by inoculating two birds each at a dose of 5 x 10^2 oocysts per bird. Inoculation of the organism was continued in the laboratory by vertical serial passages of the oocysts obtained from each bird in White Leghorn chicks for five generations. A total of 50 birds were utilised in this experiment. After five serial passages it was designated as the pure line of *E. tenella*. The oocysts and lesions produced were identified based on McDougald and Reid (1991).

At each vertical passage three uninfected birds as controls were also maintained simultaneously.

3.7 Confirmation of pure line *Eimeria tenella*

Portions of caeca and intestines of the infected birds were collected, fixed in 10 per cent formalin and processed for routine histopathological examination (Sheehan and Hrapchak, 1980) and stained for further confirmation of the species.

3.8 Standardisation of dose for infection

After conducting a few trials the dose for experimentally infecting chicks with *E. tenella* was

standardised. The sample containing pure line of *E. tenella* oocysts was stirred thoroughly to obtain a homogenised uniform suspension. Then 0.1 ml (100 µl) of the pooled sample was transferred to a glass slide and all the sporulated oocysts present within were counted under a binocular microscope. The concentration of oocysts was adjusted in such a way that 1.5 ml of the suspension contained 1.5 x 10^3 sporulated *E. tenella* oocysts.

3.9 Development of stabilised strain

This was carried out following the method of Rao *et al.* (1999). A dose of 1.5 x 10^3 sporulated oocysts of pure line *E. tenella* was administered orally to six White Leghorn male chicks of three to five days of age each, obtained from the University Poultry Farm, Kerala Agricultural University, Mannuthy. The birds were reared in wooden boxes provided with sufficient light and heat. Chick mash without anticoccidials and water was given *adlib.* The caecal contents were harvested at 168 hr pi. The oocysts thus recovered were allowed to sporulate and used in subsequent passages, for developing the stabilised strain. A total number of 90 chicks in 15 cyclical passages were made use of in this experiment.

3.10 Development of precocious strain

A precocious strain of *E. tenella* was developed simultaneously along with the development of the stabilised strain. Selection for development of a precocious strain was conducted as per the method described by Rao *et al.* (1999) with a few modifications. Sporulated pure line *E. tenella* oocysts were administered at a dose of 1.5×10^3 per bird orally to a batch of six White Leghorn coccidia free male chicks of three to five days of age.

The caecal contents were harvested at 136 hr pi and the oocysts were allowed to sporulate. Sporulated oocysts were subsequently repassaged to another group of six chicks at the same dose. The time of sacrifice of the infected chicks was predetermined and oocysts were collected from the droppings one to two hr before that time. Oocysts were harvested from the caecal contents also after sacrifice. Administration of sporulated oocysts collected after each passage to separate groups of six birds at the standardised dose was continued by reducing the time for sacrifice by one hr at each passage. Whenever oocyst collection was inadequate, that particular cyclical passage was repeated. Such repetition

was necessary at 130^{th}, 128^{th}, 126^{th} and at 125^{th} hr. As the number of oocysts obtained after slaughter at 125^{th} hr was inadequate, it was not possible to continue the experiment any further. A total of 108 birds were utilised for the above experiment.

3.11 Controls

Three birds were reared under the same coccidia free environmental conditions as controls simultaneously for each batch of experimental birds during each cyclical passage. Droppings of the control birds were examined regularly. The changes in the caecum were noted during slaughter.

3.12 Comparison of stabilised strain and precocious strain

The oocysts of the stabilised and precocious strains of *E. tenella* developed were made to sporulate. A single dose containing 2.5 x 10^3 sporulated oocysts of both strains was inoculated orally to ten, three to five day old White Leghorn male chicks maintained in two separate groups. Three uninfected control birds were reared simultaneously under the same environmental conditions. Comparison of the gross characteristics of the oocysts and pathogenesis encountered in the birds in

both the groups was studied based on the following parameters.

3.12.1 Prepatent period

The time interval between the inoculation of oocysts and the recovery of oocysts from faeces of birds of both the groups was noted.

3.12.2 Oocyst morphometry

The morphological details of both the sporulated and non-sporulated oocysts of the two groups were studied under an oil immersion objective of the microscope. The size of the oocysts were measured by micrometry. Measurements of atleast 100 oocysts were taken and the average was determined.

3.12.3 Oocyst production potential

Oocyst production potential was assessed by determining the number of oocysts per gram (OPG) of faeces at 125, 130, 135, 145, 150, 155, 160 and 168 hr pi in both the groups.

One gram of faeces was weighed and placed in a flask. Fifteen ml of water was added. The flask was corked and the contents mixed thoroughly in order to make a homogeneous mixture. The suspension was strained through

a sieve. The filtrate was stirred and 0.15 ml of the filtrate was taken by a pipette and discharged on a clean glass slide and covered with a cover slip. The whole area occupied by 0.15 ml sample was examined under low power and all oocysts were counted. This number multiplied by 100 gave the number of oocysts per gram. The average of three such counts was determined.

3.12.4 Clinical symptoms

The infected birds were kept under close observation and the clinical symptoms manifested after inoculation were noted.

3.12.5 Gross changes of caeca

Two chicks from each group infected with the stabilised and precocious strains were sacrificed on day 2, 4, 6 and 8 pi. The pathogenicity and lesion score of the caeca as described by Johnson and Reid (1970) were noted during postmortem. The lesions were scored on a 'zero' to '+4' scale according to the lesion scoring technique, where 'zero' implied a normal caeca and +4 a maximum damaged caeca.

3.12.6 Individual body weight

The individual body weight of each bird belonging to both the groups were noted on day 0, 2, 4, 6 and 8 pi and the mean body weight gain was derived.

3.12.7 Mortality

All the experimental birds were observed daily for any mortality. Mortality was recorded and expressed as per cent mortality in each group.

3.12.8 Histopathology

Portions of caeca of birds sacrificed on day 2, 4, 6 and 8 pi were collected and fixed in 10 per cent formalin and processed for routine histopathological examination as detailed by Sheehan and Hrapchak (1980). The sections were cut at 5 µm thickness and stained with haematoxylin and eosin. Whenever mortalities occurred during the course of experiment, caeca of those birds also were processed for histopathology.

4. RESULTS

4.1 Prevalence of *Eimeria tenella*

Prevalence of coccidial infections in chicken as obtained from the postmortem registers maintained at the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy are furnished in Table 1.

A total of 2938 viscera were examined out of which, *Eimeria* spp. was detected in 550 cases (18.72 per cent). Caecal coccidiosis due to *E. tenella* accounted to 242 (48.4 per cent) from the coccidia positive viscera while the overall prevalence was 8.23 per cent. Season-wise prevalence of the disease could also be observed from the table which showed highest incidence of caecal coccidiosis (21.05 per cent) during the cold wet season (June to August).

4.2 Confirmation of *Eimeria tenella*

The oocysts collected from a field outbreak of caecal coccidiosis were confirmed to be that of *E. tenella* by observing the following parameters.

4.2.1 Sporulation time

The sporulation time of the oocysts was found to be 24 to 36 hr.

Table 1. Prevalence of coccidiosis in chicken

Sl. No.	Season	Month	No. of viscera examined	No. positive for coccidiosis	Percent positive	No. positive for caecal coccidiosis	Percent positive
1	Cold wet South west monsoon (Heavy rainfall)	June-Aug	399	100	25.06	84	21.05
2	Warm wet North east monsoon (low rainfall)	Sep-Nov	1198	299	24.90	72	6.01
3	Dry	Dec-May	1341	151	11.26	86	6.41
Total			2938	550	18.72	242	8.23

4.2.2 Oocyst morphology and micrometry

Oocysts were broad, ovoid without a micropyle and measured 22.6 µ (19.2 to 26 µ) x 19 µ (16 to 22µ). The wall was double contoured. A polar granule was often present in the oocyst. Refractile globule was present in the sporozoites. Sporocysts measured 11µ (9 to 13 µ) by 7 µ (5 to 9 µ) and the sporozoites were elongated.

4.2.3 Prepatent period

The prepatent period of the field strain of *E. tenella* was found to be 146 hr experimentally.

4.3 Single oocyst isolation technique

Of the twelve chicks inoculated with a single oocyst of *E. tenella*, five chicks developed the

infection. At 146 hr pi, the oocyst per gram (OPG) of faeces obtained was $2x10^2$ to $2.5x10^2$ and that of the caecal contents was $5x10^2$ to $7x10^2$.

4.4 Propagation of pure line of *Eimeria tenella*

The oocysts obtained from each bird were amplified by inoculating two birds each at a dose of $5x10^2$ oocysts per bird. At 146 hr, the OPG recorded from each infected bird was approximately $2x10^5$. Finally after five serial passages vertically in a total of 50 White Leghorn chicks, these oocysts were designated as the pure line of *E. tenella*.

4.5 Confirmation of pure line *Eimeria tenella*

Histopathological examination revealed the presence of schizogonic stages and gametogonic stages only in the caecal epithelium, thereby confirming the specificity of *E. tenella*.

4.6 Standardised dose of *Eimeria tenella*

A concentration of $1.5x10^3$ sporulated oocysts of *E.tenella* resulted in a mean OPG of $6x10^5$ in the faeces and $8x10^5$ in the caecal contents at 146 hr on experimental infection.

4.7 Development of stabilised strain

A dose of $1.5x10^3$ sporulated oocysts of *E. tenella* was administered orally to six White Leghorn male chicks, each of three to five days of age. The caecal contents were harvested at 168 hr pi. The mean OPG of faeces of the birds was found to be $6x10^5$ to $7x10^5$ and that of caecal contents was $8x10^5$ to $1x10^6$. After 15 cyclical passages of the sporulated oocysts in White Leghorn chicks, consisting of six chicks per passage, the oocysts recovered were designated as the stabilised strain of *E. tenella*.

4.8 Development of precocious strain

The caecal contents harvested at 136 hr pi revealed a mean OPG of $9.8x10^3$. The oocysts collected at 125^{th} hr pi i.e. after 18 passages including repetitions were designated as the precocious strain. The oocyst output at each passage are given in Table 2.

4.9 Control birds

Droppings of the control birds were found to be free of oocysts. No lesions were observed in the caecum either, during slaughter.

Table 2. Development of a precocious line: Oocyst output after administering $1.5x10^3$ oocysts per bird

Hr pi	OPG faeces	OPG caeca
146	6×10^5	8×10^5
136	-	9.8×10^3
135	6.5×10^3	9×10^3
134	4×10^3	6×10^3
133	4.4×10^3	5.8×10^3
132	3.1×10^3	4.1×10^3
131	2.3×10^3	3.5×10^3
130	6×10^2	1.1×10^3
130	2×10^3	3×10^3
129	1.2×10^3	3×10^3
128	5.5×10^2	2×10^3
128	5×10^2	2.8×10^3
127	2×10^3	3.1×10^3
126	6×10^2	1.8×10^3
126	6.2×10^2	2.1×10^3
126	2.1×10^3	3×10^3
125	5×10^2	2.2×10^3
125	5.5×10^2	2.5×10^3
125	2.3×10^3	3.1×10^3

4.10 Comparison of stabilised strain and precocious strain

Variations in the parameters mentioned below, between the stabilised and precocious strain infected groups were noted and compared.

4.10.1 Prepatent period

The prepatent period of the stabilised strain of *E. tenella* was found to be 135 hr while that of the precocious strain was 125 hr after infection.

4.10.2 Oocyst morphometry

There were no marked changes in the oocyst morphology of both the strains.

Measurements (length x width) of the stabilised strain were the following.

Oocysts : 22.6 µ (19.2 to 26 µ) x 19.23 µ (16 to 22 µ)

Sporocyst : 11 µ (9 to 13 µ) x 7 µ (5 to 9 µ)

The shape index (length/width) was 1.18 (Fig.1).

The oocysts of the precocious strain measured 21.8 µ (18.6 to 26.1 µ) x 18.25 µ (15 to 21.5 µ). The sporocysts were 10.2 µ (7.5 to 13.5 µ) x 6.7 µ (4.5 to 7.5 µ). The shape index was 1.19 (Fig.2).

4.10.3 Oocyst production potential (Table 3)

The oocyst production potential assessed by counting the oocysts per gram (OPG) of faeces was as follows:

Table 3. Oocyst production potential

hr pi	Stabilised strain infected group	Precocious strain infected group
125	Zero	2.5×10^3
130	Zero	3.2×10^3
135	4.3×10^3	7×10^3
145	6×10^5	5.5×10^4
150	6.2×10^5	7×10^4
155	7×10^5	1×10^5
160	7×10^5	2×10^5
168	7×10^5	2×10^5

The mean OPG of faeces of the stabilised strain infected group was nil till 130th hr pi which was followed by gradual increase. On the other hand, the precocious strain infected group showed a wide range of OPG during the initial hours of infection. Later on, it was noted that the mean OPG became less when compared with that of the stabilised strain.

4.10.4 Clinical symptoms

Clinical signs were very severe in the birds infected with the stabilised strain. Large amounts of blood was detected in the droppings from day 4 to 6 pi. In majority of birds, blood was seen to spurt directly from the cloaca. Faecal matter was found very less in such cases. Feathers around the vent was soiled with blood stained droppings. Feed intake was drastically reduced and the birds were very dull, droopy, emaciated and unthrifty (Fig. 3).

The birds infected with precocious strain showed very mild clinical signs. Only very little blood was noticed in the droppings examined on day 4 to day 6 pi. Feed intake was not affected at all. The birds were active (Fig.4).

Control birds showed no clinical signs.

4.10.5 Gross changes of caeca

Day 2 pi

There was apparently no difference between the caeca of birds infected with either the stabilised or precocious strains. A few petechial haemorrhages were

noticed on the caecal wall of the stabilised strain infected group.

Day 4 pi

The caeca of birds infected with stabilised strain were engorged and bright red in colour. Caecal wall was greatly thickened. The lumen was filled with blood, blood clots and debris. Marked haemorrhagic spots were observed on the caecal wall (Fig.5).

The caeca of the birds infected with precocious strain appeared slightly swollen and were light reddish in colour. Caecal wall was somewhat thickened. Caecal contents were slightly blood tinged. A few haemorrhagic spots were noticed on the caecal wall (Fig. 6).

Day 6 pi

The lesions of the stabilised strain and precocious strain infected group were similar to those that occurred on day 4 pi. But the lesions of the precocious strain were less intense.

Day 8 pi

In the stabilised strain infected group, a consolidated caseous plug was seen to fill the lumen completely. The caecal wall was very much thickened while

the caeca and caecal contents of the precocious strain infected group appeared normal except for a slight thickening of the caecal wall. The lesions noticed were scored as follows.

Stabilised strain infected group : +4
Precocious strain infected group : +2
Postmortem of the control birds showed normal and healthy caeca.

4.10.6 Body weight

The individual body weight of the birds belonging to each group was noted on day 0, 2, 4, 6 and 8 pi (Table 4).

Table 4. Mean body weight (g)

Day pi	Stabilised	Precocious	Control	F value	Remarks
Day 0	42.70 ± 0.95	43.20 ± 0.80	46.33 ± 1.45	2.042	NS
Day 2	44.90 ± 1.04^{a}	46.50 ± 0.83^{a}	50.66 ± 1.76^{b}	4.280	*
Day 4	46.80 ± 1.46^{b}	50.50 ± 0.42^{a}	56.00 ± 1.53^{c}	15.460	**
Day 6•	45.5	52.4	62.6	-	-
Day 8•	♦	55.0	70.3	-	-

* Significant at 5% level

** Significant at 1% level

Means bearing common letter in the superscript row-wise do not differ significantly

• Analysis could not be done due to insufficient number of chicks as a result of mortality

♦ Due to mortality body weight could not be recorded

Increase in the body weight was observed to be very low in the stabilised strain infected group when compared to both the precocious strain infected group and the controls.

In the precocious strain infected group there was a moderate increase in the mean body weight throughout the period of experiment but it was less when compared to that of the control birds.

On day 6 pi there was a mean weight gain of 2.8 g, 9.2 g and 16.3 g from the initial body weight in the stabilised, precocious and control groups, respectively.

Analysis of the initial body weight (day 0) of the experimental birds statistically showed no significant difference between the three groups before infection. On day 2 pi, the difference in the body weights, between the three groups was significant at 5 per cent level and on day 4 pi, significance at 1 per cent level was noted (Table 4).

4.10.7. Mortality

In the group infected with stabilised strain, there was 40 per cent mortality, whereas there was only 10 per

cent mortality in the group infected with precocious strain. No mortality was recorded in the control group.

4.10.8 Histopathology

Results of the histopathology of the portions of caeca of both the stabilised and precocious strain infected groups are summarised in Table 5.

In the stabilised strain infected group, the first generation schizonts were seen in the epithelial cells of the deeper parts of lamina propria at 48 hr pi (Fig.7). Mature second generation schizonts with closely packed banana shaped merozoites were visible at 96 hr pi (Fig.9). A few gametocytes along with mature second generation schizonts were noticed at 120 hr pi (Fig.11). There was degeneration of the epithelial cells and presence of inflammatory cells and erythrocytes in the lamina propria. Oocysts were first noted at 144 hr pi in this group (Fig. 13).

In the precocious strain infected group the first generation schizonts were seen in the epithelial cells of the superficial parts of lamina propria at 48 hr pi (Fig.8). The mature second generation schizonts with banana shaped merozoites appeared as early as 72 hr pi

(Fig.10). Gametocytes were noticed at 96 hr pi (Fig.12) and oocysts first occurred at 120 hr pi (Fig.14) which was about 24 hr earlier than their appearance with the stabilised strain infection.

Table 5. Endogenous development of parasites of stabilised and precocious strains in chicken caeca

Hr pi	Stabilised strain infected group	Precocious strain infected group
48	1st generation schizonts	1st generation schizonts
72	1st generation schizonts	Mature 2nd generation schizonts
96	Mature 2nd generation schizonts	Mature 2nd generation schizonts and a few gametocytes
120	Mature 2nd generation schizonts and a few gametocytes	Gametes and oocysts
144	Gametes and oocysts	Gametes and oocysts
192	Gametes and oocysts	Gametes and oocysts

Figures

STABILISED STRAIN

Fig. 1

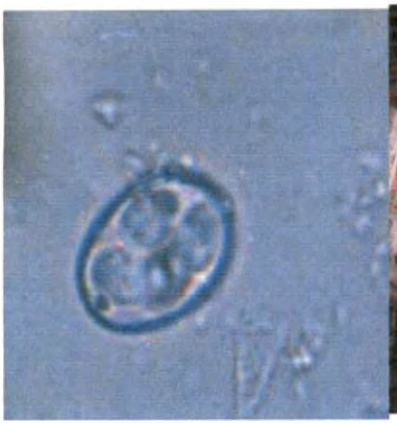


Fig. 3

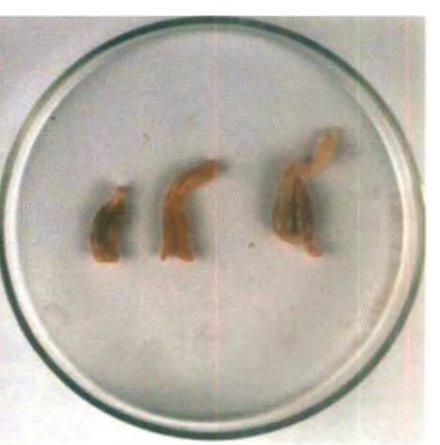
Fig. 5

PRECOCIOUS STRAIN

Fig. 2

Fig. 4

Fig. 6



Figures

7 First generation schizonts of stabilised strain infected group, seen in the epithelial cells of the deeper parts of lamina propria at 48 hr pi (H & E x 400)

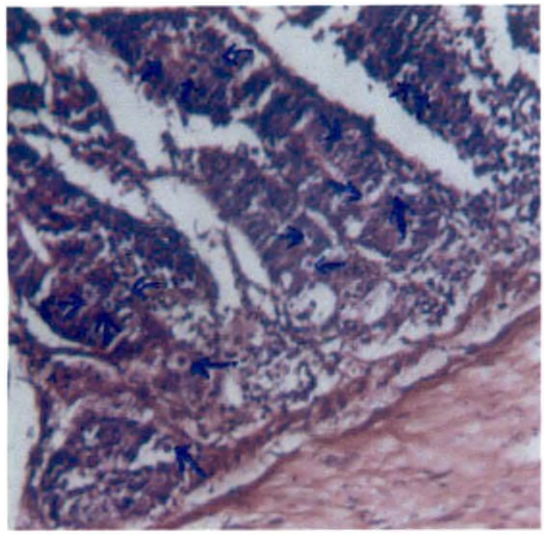
8 First generation schizonts of precocious strain infected group, seen in the epithelial cells of the superficial parts of lamina propria at 48 hr pi (H & E x 400)

9 Mature second generation schizonts of the stabilised strain infected group at 96 hr pi (H & E x 400)

10 Mature second generation schizonts of the precocious strain infected group at 72 hr pi (H & E x 400)

STABILISED STRAIN

Fig. 7



PRECOCIOUS STRAIN

Fig. 8

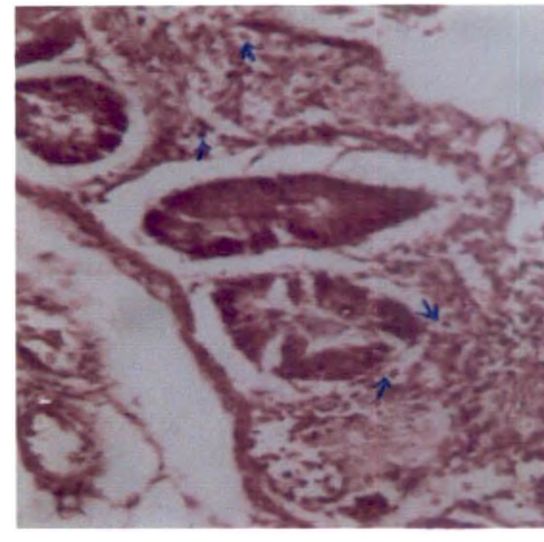


Fig. 9

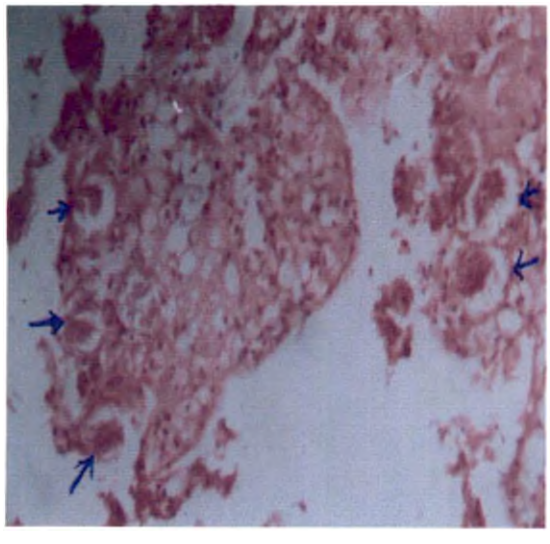
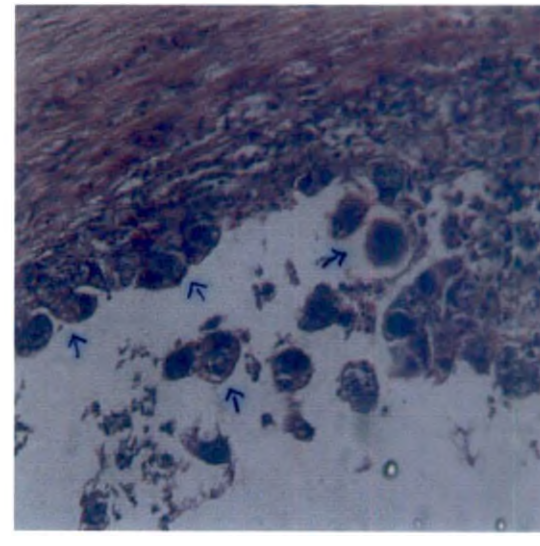


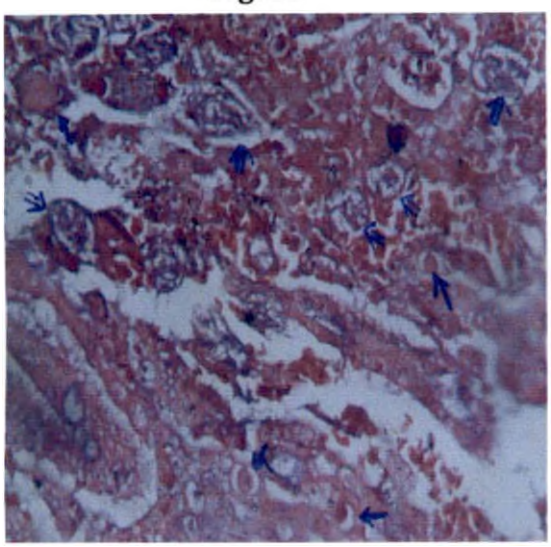
Fig. 10



Figures

STABILISED STRAIN

Fig. 11



PRECOCIOUS STRAIN

Fig. 12

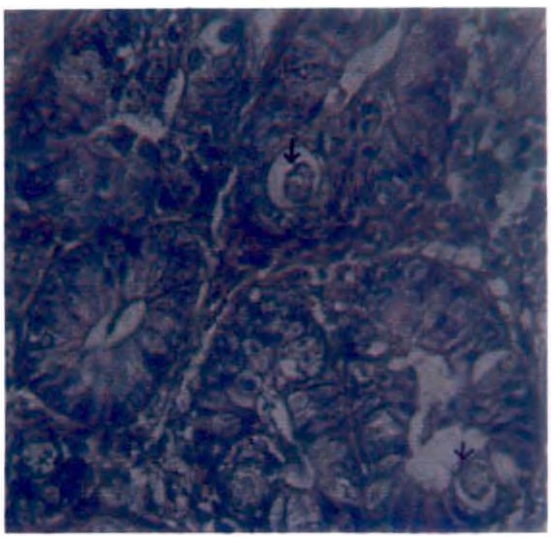


Fig. 13

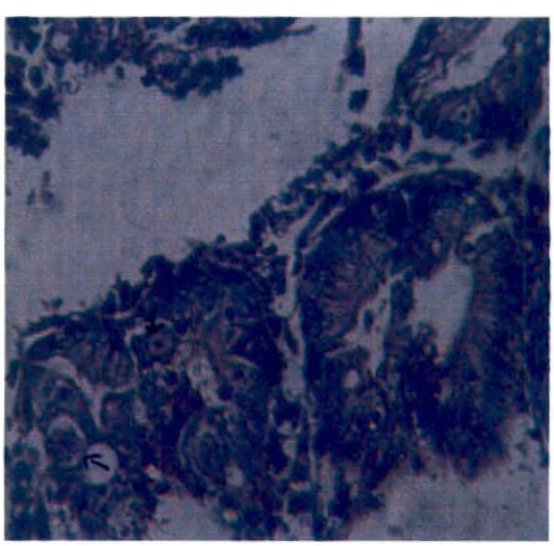
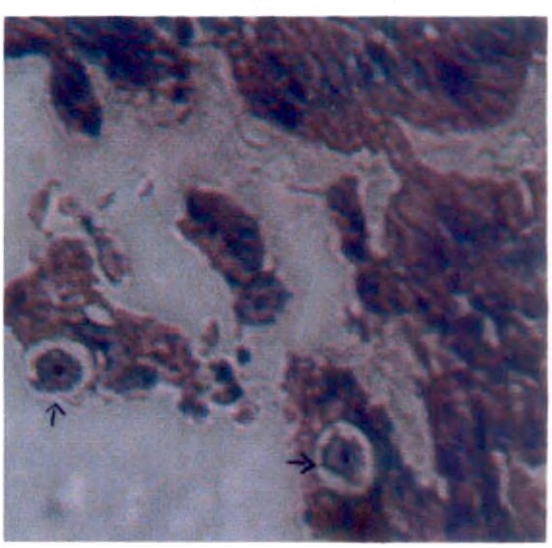


Fig. 14



5.DISCUSSION

5.1 Prevalence of *Eimeria tenella*

In the present study, 550 cases of coccidiosis were reported from 2938 viscera examined as per the registers maintained in the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, during the period from June 2000 to May 2001.

Caecal coccidiosis occurred in 242 cases resulting in a prevalence of 48.4 per cent. The results indicate that *Eimeria tenella*, the causative organism of caecal coccidiosis was highly prevalent among the eimerian organisms affecting chicken.

Jagadish Babu *et al.* (1976), Srinivasa *et al.* (1989), Rana and Tikaram (1994), Panda *et al.* (1996) and Gigi George (1997) had also reported *E. tenella* as the most prevalent species among the eimerian organisms, the rate of prevalence ranging from 28.9 to 72 per cent.

The present prevalence studies also detected a higher seasonal prevalence (21.05 per cent) of the organism during June to August (cold wet season). Similar findings were reported by Jahurulkarim *et al.* (1994) and Panda *et al.* (1996).

Higher prevalence of coccidiosis during the cold wet season could be due to the heavy rainfall and high humidity which cause stress in birds and are greatly conducive for the development and propagation of the infection.

5.2 Confirmation of species

The organisms obtained from a field outbreak of caecal coccidiosis was confirmed as *E. tenella* by determining the various criteria specific to it as described by Joyner and Long (1974).

5.3 Single oocyst isolation

Five birds successfully developed *E. tenella* infection when twelve birds were inoculated with a single oocyst of the organism. This amounted to a patency of 41.6 per cent. Long *et al.* (1976) noted that 30 to 50 per cent of single oocyst infected birds developed patent infections.

5.4 Confirmation of pure line *Eimeria tenella*

Histopathological confirmation of pure line *E. tenella* by noting the schizogonic stages only in the caeca is a very specific character as reviewed by Jagadishbabu *et al.* (1976).

5.5 Development of stabilised strain

The stabilised strain was developed as per the method described by Rao *et al.* (1999) who had used $3x10^3$ sporulated *E. tenella* oocysts per bird for subsequent passages. However, in this experiment this dose was found to cause heavy mortality in chicks. Hence after a few trials, the dose was standardised to $1.5x10^3$ sporulated oocysts and the stabilised strain was developed after 15 cyclical passages. Oocysts for setting up infection were collected at 168 hr pi after each passage as adopted by Jeffers (1975) and Rao *et al.* (1999).

5.6 Development of precocious strain

The precocious strain of *E. tenella* was developed as per the method of Rao *et al.* (1999) with slight modifications.

Birds of three to five days of age were used in this experiment as described by Montes *et al.* (1998) so that the risk of cross contamination during their rearing period was brought to a minimum.

During the serial passages of the parasites, Rao *et al.* (1999) collected the oocysts about six hr after the first finding of oocysts in the faeces. But in the

present experiment, oocysts were collected directly from the caecal contents of the birds sacrificed at a predetermined time after infection. This enabled a more precise determination of the prepatent period and avoided the processing of large bulk of faeces.

At 136 hr pi the OPG of the caecal contents reduced to $9.8x10^3$ which was very less, compared to the harvest at 146 hr pi ($8x10^5$). These oocysts were the early developed ones and were selected and repassaged.

The selection was continued applying selection pressure in increments of one hour reduction per passage.

Repetition of passages was essential at the 130^{th}, 128^{th}, 126^{th} and 125^{th} hr pi to get adequate oocysts as pointed out by Rao *et al.* (1999) who also relaxed the selection pressure in four different generations. Although Jeffers (1975), Kawaguchi *et al.* (1988) and Rao *et al.* (1999) were able to develop a precocious strain of *E. tenella* with a prepatent period of 12 to 24 hr shorter than that of the parent strain, in the present study the precocious strain of *E. tenella* was designated after 18 passages with a reduction of the prepatent period from 146 to 125 hr. Insufficient number of oocysts recovered

after the 125th hr curtailed further progress of the experiment. Since the time for the development of oocysts was reduced with the reduction in prepatent time, the amount of oocysts recovered was found to decrease at each step.

5.7 Control birds

Absence of oocysts in the faeces and lesions in the caeca of the control birds confirmed that there has been no extraneous infection and that the infection produced in the experimental birds were only due to the experimentally inoculated oocysts.

5.8 Comparison of the stabilised strain and precocious strain

5.8.1 Prepatent period

The prepatent period of the stabilised strain was found to be 135 hr in the present study and more or less concurs with the findings of Rao *et al.* (1999). The oocysts that were found as early as 135 hr might be the early developed ones which get selected by themselves after repeated cyclical passages.

Prepatent period of the precocious strain was still shorter i.e. 125 hr. This is 21 hr less than that of the parent strain.

The precocious strains of *E. tenella* developed by Jeffers (1975), Kawaguchi *et al.* (1988) and Rao *et al.* (1999) showed a prepatent period of 125, 112 and 120 hr, respectively.

5.8.2 Oocyst morphometry

Jeffers (1975) stated that the increase in the developmental rate of the precocious strain was also accompanied by a reduction in oocyst size. He suggested that the reduction in oocyst dimensions of the precocious strain was not a direct result of selection for precociousness but rather a function of time permitted for growth of macrogametes.

Contrary to the above statement, in the present study, no significant difference was noted between the oocysts of both the strains as observed by Rao *et al.* (1999).

5.8.3 Oocyst production potential

The results indicated that in the initial hours after infection, the oocyst output of the precocious strain was higher than the stabilised strain concurring with the findings of Jeffers (1975) and Rao *et al.*

(1999). This is attributed to the selection for early development of oocysts.

It is noted that at later hours after infection, the precocious strain produced comparatively less number of oocysts than the stabilised strain which must be due to its precocity.

The precocious strain has a reproductive potential inherently smaller than its parent wild strain and as a consequence is less pathogenic.

5.8.4 Clinical symptoms

The results obtained in the present study were in accordance to the observations of Jeffers (1975), Kawaguchi *et al.* (1988) and Rao *et al.* (1999).

Severe symptoms like massive haemorrhage and total destruction of epithelial cells seen in the group infected with the stabilised strain may be due to its higher reproductive potential whereas mild infections observed in the group infected with precocious strain could be due to its reduced reproductive potential in comparison to the stabilised strain.

5.8.5 Gross changes of caeca at autopsy

Drastic and significant changes were noted on the caeca of the birds infected with stabilised strain (lesion score: +4), while only mild changes were noted on the caeca of birds infected with precocious strain (lesion score: +2).

Similar results were observed by Jeffers (1975), Kawaguchi *et al.* (1988) and Rao *et al.* (1999).

5.8.6 Body weight

The body weight gain was very less in the stabilised strain infected group and moderately less in the precocious strain infected group compared to the control group. This was due to the severity of infection and reduced feed intake in the stabilised strain infected group and a comparatively lower rate of infection in the precocious strain infected group.

Jeffers (1975), Kawaguchi *et al.* (1988) and Rao *et al.* (1999) also noted that the adverse effects of the parasite infection on the body weight gain were relatively greater in the stabilised strain infected group than in the precocious strain infected group.

5.8.7 Mortality

Reduced pathogenicity, reduced oocyst production and subsequent less damage to the caecal epithelium resulted in reduced mortality in the precocious strain infected group when compared to the stabilised strain infected group.

5.8.8 Histopathology

The histological observations of the endogenous development of the parasite showed distinct differences between the stabilised and the precocious strain in the rate of development and site of occurrence. There was a difference of about 24 hr between the stabilised strain and the precocious strain infected groups in the appearance of various stages.

The second generation schizogony proceeded at a much faster rate in the precocious strain resulting in the appearance of mature second generation schizonts at 72 hr pi while they were noted only at 96 hr pi in the stabilised strain. Gametocytes and oocysts were found as early as 96 hr and 120 hr pi respectively in the precocious strain infected group whereas they were found at 120 hr pi and 144 hr pi in the stabilised strain

infected group. Similar observations were noted by Jeffers (1975).

The developmental stages occurred in the epithelial cells of the superficial parts of lamina propria in the precocious strain while in the stabilised strain, they were seen in the deeper parts of lamina propria. Such results were recorded by Kawaguchi *et al.* (1988) also.

The concept of attenuation by a genetic selection process for development of precocious strain is known for the last two decades. If attenuated lines of all the species of *Eimeria* which infect the chicks could be developed it would then be possible to determine whether a multivalent attenuated vaccine against coccidiosis would be effective in commercial poultry houses. Of special interest is whether the vaccine lines protect against the antigenically diverse strains present in the field. Precocious strains, apart from their obvious potential as constituents of a live attenuated vaccine against coccidiosis in chicks, could also prove to be useful in the study of the biology of *Eimeria* spp.

SUMMARY

6. SUMMARY

1. The present study was undertaken with the objective to develop a precocious strain of *Eimeria tenella*, the causative organism of caecal coccidiosis in chicken and to compare its characteristics with that of the stabilised strain developed simultaneously.

2. As an insight to the gravity of caecal coccidiosis in chicken, the prevalence of *E. tenella* was also studied from the postmortem registers maintained at the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, during the period from June 2000 to May 2001. The prevalence was found to be 48.4 per cent out of 550 coccidia positive viscera, with a higher rate of infection (21.05 per cent) noted during the cold wet season (June to August).

3. Oocysts of *E. tenella* were isolated from a typical field case of caecal coccidiosis of chicken. A pure line of *E. tenella* was established in the laboratory by single oocyst isolation technique in 12 birds of three to five days of age, reared in wooden boxes, following standard managemental conditions. The prepatent period of the pure line was determined as 146 hr. The

sporulation time for the oocysts was 24 to 36 hr. At post mortem the lesions were found only in caecal pouches. Histopathology revealed schizogonic stages only in the caeca confirming it to be a pure line of *E. tenella*.

4. Development of stabilised strain

A dose of $1.5x10^3$ sporulated pure line *E.tenella* oocysts was administered orally to six, three to five day old coccidia free White Leghorn chicks reared in wooden boxes under standard managemental conditions. Three control birds were reared under similar environmental conditions. The caecal contents were harvested at 168 hr pi. The oocysts thus harvested were allowed to sporulate and used in subsequent passages at the same dose and in the same number of chicks. At the end of 15 cyclical passages the oocysts were designated as the stabilised strain of *E. tenella*.

5. Development of precocious strain

A dose of $1.5x10^3$ sporulated pure line *E. tenella* oocysts per bird was administered orally to six, three to five day old coccidia free White Leghorn chicks reared in wooden boxes under standard managemental conditions. Three control birds were reared under similar

environmental conditions. The caecal contents of the infected birds were harvested at 136 hr pi. The oocysts thus obtained were allowed to sporulate and subsequently repassaged by applying selection pressure in increments of one hour reduction at each passage. Whenever the oocysts collected were inadequate the passage was repeated. Such repetitions were necessary at 130^{th}, 128^{th}, 126^{th} and 125^{th} hr pi. After 18 cyclical passages, the oocysts recovered at 125 hr pi which was 21 hr less than the original prepatent period of the parent strain were designated as the precocious strain of *E. tenella*. It was not possible to continue the experiment any further as the number of oocysts obtained were inadequate after 125 hr pi.

6. Comparison of the stabilised and precocious strain

A dose of $2.5x10^3$ sporulated oocysts each of the precocious and stabilised strain of *E. tenella* were administered orally to ten, three to five day old White Leghorn chicks reared in separate groups and the following observations were made.

The prepatent period of the precocious strain was observed as 125 hr and that of the stabilised strain as 135 hr. Reduction in the time of patency in the case of

precocious strain to 125 hr can be attributed to repeated selection for the early development of oocysts.

The oocyst morphometry did not reveal any significant difference between the two strains.

The oocyst production potential of the precocious strain was higher than that of the stabilised strain during the early hours of infection due to its precocity. During the later stages, the oocyst count reduced in the precocious strain infected group due to the loss of its reproductive potential.

The precocious strain infected birds showed milder clinical signs and less lesion scores of caeca at autopsy and less mortality when compared to the stabilised strain infected group.

The adverse effects of the parasite infection on body weight gain were relatively less in the precocious strain infected group than in the stabilised strain infected group.

All the above findings could be attributed to the reduced reproductive potential and reduced pathogenicity of the precocious strain.

Endogenous study revealed an accelerated growth in the early stages of the asexual life cycle in the precocious strain infected group. Mature second generation schizonts were found as early as 72 hr pi in contrast to 96 hr pi in the stabilised strain infected group. The parasites were found in the more superficial areas of the lamina propria in the precocious strain infected group.

The characteristics of the precocious strain like reduction in prepatent time, reproductive potential and pathogenicity contribute to its usefulness as a successful vaccine candidate.

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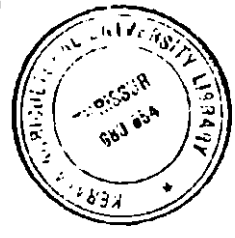

DEVELOPMENT OF A PRECOCIOUS STRAIN OF *Eimeria tenella*

By

SANGEETHA. R.

ABSTRACT OF A THESIS

Submitted to the Kerala Agricultural University
in partial fulfilment of the requirements
for the degree of

Master of Veterinary Science
in
Veterinary Parasitology

COLLEGE OF VETERINARY AND ANIMAL SCIENCES
KERALA AGRICULTURAL UNIVERSITY
MANNUTHY, THRISSUR - 680651
KERALA, INDIA

2001

ABSTRACT

Studies on the prevalence of caecal coccidiosis in chicken, by referring to the postmortem registers maintained at the centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy revealed that 48.4 per cent of 550 coccidia positive viscera was due to *Eimeria tenella*. A pure line of *E. tenella* was established in the laboratory by single oocyst isolation technique from a virulent field case of caecal coccidiosis in chicken. A stabilised strain of *E. tenella* was developed by administering a dose of $1.5x10^3$ sporulated pure line *E. tenella* oocysts per bird orally to six three to five day old coccidia free White Leghorn chicks and harvesting the caecal contents at 168 hr pi for 15 cyclical passages. For the development of a precocious strain of *E. tenella* a dose of $1.5x10^3$ sporulated *E. tenella* oocysts per bird was administered orally to six, three to five day old White Leghorn chicks. The caecal contents were harvested at 136 hr pi and the oocysts were allowed to sporulate and subsequently repassaged by applying selection pressure in increments of one hour reduction at each passage. At the end of 18 cyclical passages, oocysts obtained at 125 hr

pi were designated as the precocious strain of *E. tenella*.

A comparative study between the precocious and stabilised strain of *E. tenella* revealed the following observations.

The prepatent period of the precocious strain was 125 hr while that of the stabilised strain was 135 hr. The oocyst morphology did not reveal any significant difference between the two strains. The precocious strain was less pathogenic and produced milder clinical signs than the stabilised strain. The gross lesions of caeca were less morbid in the precocious strain infected group. Weight gain was more in the precocious strain infected group than in the stabilised strain infected group. The precocious strain produced less mortality and showed a drop in oocyst production.

Endogenous study revealed an accelerated growth in the early stages of the asexual life cycle in the precocious strain infected group.