Studies on Resistance of Some Chemical Compounds Against Kimeria Tenella Field Isolate



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SUBMITTED TO THE

## RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF POST - GRADUATE STUDIES)
PUSA (SAMASTIPUR) BIHAR

In partial fulfilment of the requirements
FOR THE DEGREE OF

Master of Veterinary Science

(VETERINARY PARASITOLOGY)

By

Dr. Suman Kumar Registration No. - M/V Para/80/2003-2004

Department of Veterinary Parasitology
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#### **CERTIFICATE-I**

This is to certify that the thesis entitled "Study on Resistance of Some Chemical compounds Against Eimeria tenella Field Isolate" submitted in partial fulfilment of the requirements for the Degree of Master of Veterinary Science (Veterinary Parasitology) of the faculty of post-graduate studies, Rajendra Agricultural University, Pusa, Samastipur, Bihar is the record of bonafide research work carried out by Dr. Suman Kumar Registration No. M/V.Para/80/2003-04, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that the assistance and help received during the course of this investigation and preparation of the thesis have been fully acknowledged.

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Major Advisor.

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#### **CERTIFICATE - II**

We the undersigned members of the Advisory Committee of **Dr. Suman Kumar**, Registration No. – M/V. Para / 80 / 2003 – 2004, a candidate for the Degree of Master of **Veterinary Science** with Major in **Veterinary Parasitology** have gone through the manuscript of the thesis and agree that the thesis entitled "Study on Resistance of Some Chemical Compounds Against *Eimeria tenella* Field Isolate" may be submitted by **Dr. Suman Kumar** in partial fulfilment of the requirements for the degree.

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Dedicated

to my

Late

Mother



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B.V.C., Patna

Date: ...../2006

Suman Kumar

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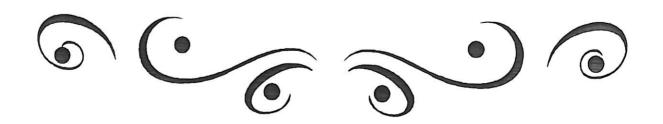
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CHAPTER - I

## INTERIOR CON



## INTRODUCTION

In India, poultry farming was a backyard venture almost up to 1960 but entire scenario has changed during last four decades and now it is recognised as an organised and science based industry and a potential tool to fight against poverty and malnutrition. During this brief period, the poultry industry has reached a stage of self-sufficiency and sophistication in the production of feed, pharmaceuticals, vaccine, equipment and breeding stock to meet the current and future need. Poultry industry has made substantial contribution to our economy by generating both direct and indirect employment opportunities for weaker section of society, particularly to small, marginal farmers and landless labourers. India ranked 5th in annual egg production and 18th in broiler production in the year 2002-2003 (India 2003).

The annual grown rate of egg and broiler production has gone up to 4 to 6 per cent and 8 to 10 per cent respectively. Similarly the poultry meat production in estimated at 0.598 million tonne which accounts only 12 per cent of total meat production from all sources (India 2003, Indian economic survey 2002-2003). As per the estimate the poultry production in India is likely to reach 400 million broiler and 34 billions eggs per year in near future.

The value output from poultry industry in India is about 10,234 crores of rupees. Poultry industry in India generated employment opportunities for about 7.8 lakh people. It is presumed that there will be a big gap between production and

requirement of poultry products in India to meet nutritional requirements (84 gm of meat and half an egg per day per person as per the recommendation of nutritional advisory committee, Govt. of India, Panda 1970) of more than 100 crore population of India. Hence it will be necessary to produce better quality of poultry products in sufficient amount.

Growth in poultry industry and rise in poultry population also has augumented the health problems in birds. The young chicks are highly suecptable to various infectious and noninfectious diseases. Among these the greatest threat to poultry industry comes from poultry coccidiosis caused by Eimeria species which stand next to salmonellosis in causing severe economic loss to poultry industry. Coccidiosis is considered to be one of the devastating diseases in poultry all over the world. It occurs as an outbreak especially in younger stock resulting heavy mortality and morbadity. The economic loss caused by coccidiosis to poultry industry through out the world is estimated to be half of the expenditure made in feed. The loss due to coccidiosis, including cost of prophylactic agent, estimated to be 1.5 billion dollar per year and this accounts for 10 percent of total investment made in poultry industry. Though coccidiosis can effect birds of any age group, chicks up to eight weeks of age are comparatively more succeptible to infection especially in the present method of intensive farming. Different species of Eimeria affect birds in certain age groups and in a particular portion of the intestine. Young chicks aged about 5 to 21 days are more suceptable to Eimeria tenella infection but Eimeria necatrix effect chicks of

4 to 8 weeks of age, whereas *Eimeria acervulina* infects birds of still older age. The disease is more prevalent in Bihar especially during rainy season due to hot and humid climate. In order to have judicious use of anticoccidial in control programmes, continuous administration of low level of anticoccidial drug as feed additive is now under practice through out the world. The major problem in the usage of coccidiostats leads to the undesirable consequence of drug resistance (Gill and Bajwa, 1979; Surya Kumari, 1996) in the parasite against the drug leading to gradual increase in dose making it economically unviable. It is therefore, regular screening of drug is necessary to find out the most suitable and cost effective drug against the parasite at different geographical locations.

#### Objectives of investigation:

Considering the above mentioned fact and its paramount importance the present study has been designed with the following objectives:

- (I) To isolate the drug resistant strain of Eimeria tenella from field.
- (II) To screen some chemical coccidiocidal compounds against the resistant strain of *Eimeria tenella* in poultry.

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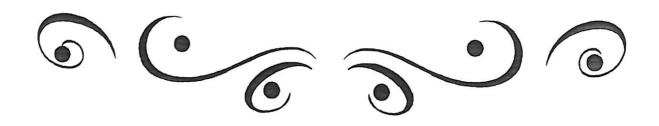




CHAPTER - II

## REVIEW

# LITERATURE



## REVIEW OF LITERATURE

#### History of anticoccidials:

Control of coccidiosis in poultry was attempted of prior to 1940s by using various kinds of alchemic recipes, skimmed milk, vinegar, and flower of sulfer. Discovery of sulfonamide as potent anticoccidial activity in early 1940s, transformed treatment of avian coccidiosis. With the remarkable growth of poultry industry has come a steady procession of chemical agents used to combat coccidiosis.

Anticoccidial compound	First approval by FDA	Action	Drug withdraw (Days before slaughter)
Sulfonamide	1940		10
Sulfamethionine	1947	Coccidiocidal	10
Sulfaquinoxaline	1948	Coccidiocidal	10
Niprofurazone	1948	Coccidiocidal	5
Arsanilic acid or sodiumorsanilate	1949	-	5
Butynorate	1954	Coccidiostat	28
Nicarbozine	1955	Coccidiocidal	4
Furazolidone	1957	Coccidiocidal	5
Mitromide + Sulfanitran + roxarasone	1958	Coccidiocidal	5
Oxytetracyclin	1959	Coccidiostat	3
Amporlium	1960	Coccidiocidal	0
Chlortetracyclin	1960	Coccidiostat	(See feeding

			restrict- tions)
Zoalene	1960	Coccidiocidal	(Higher levels, 5 days)
Amprolium+ethopabate	1963	Coccidiocidal	0
Buquinolate	1967	Coccidiostat	0
Sodium sulfachloropyrazine monohydrate	1967	Coccidiocidal	4
Sulfadine thoxine	1968	Coccidiocidal	5
Clopidol or meticlorpindol	1968	Coccidiostat	0 day at 0.0125%; 5 days at 0.025 percent.
Decoquinate	1970	Coccidiostat	0
Sulfadimethoxine + ormetoprion	1970	Coccidiocidal	5
Menensin	1971	Coccidiostat	0
Robenidine	1972	Coccidiocidal	5
Lasalcid	1976	Coccidiocidal	3
Salinomycin	1983	Coccidiocidal	0
Halofuginone	1987	Coccidiocidal	5
Narasin	1988	-	0
Madurimicin	1989	Coccidiostat	5
Narasin+nicarbozine	1989	Coccidiocidal	5
Toltrazuril	1985	Coccidiocidal	_
Diclazuril	1998	Coccidiostat	-

#### Prevalence of drug resistant strain of coccidia in poultry:

Cuckler and Malanga (1955), first reported the development of resistance in coccidian parasite to drug. They developed sulphaquinoxalin resistant strain of Eimeria experimentally after 15 serial passage. Experimentally induced

- resistance reported against anticoccidials like nitrofurazone (Joyner, 1957; Horton and Smith, 1962) suphaquinoxaline (Horton and Smith, 1962), zoalene (McLoughlin and Gordiner, 1962) and Quinolines (Mc Manus *et al.*, 1968).

## Anticoccidial used in chemotherapy of caecal coccidiosis: (a) Amprolium:

Prasad et al. (1986) studied the weight gain of poultry during amprolium therapy. They found that there was significant increase in body weight of chicks, which were treated with amprolium at therapeutic dose but as the dose increased from therapeutic level, weight gain dropped.

Evaluation of the prophylactic activity of amprolium against *Eimeria. tenella* was conducted by Gomez *et al.* (1985) Amprolium at the level of 125 ppm in feed was given to groups of 22-day-old white leghorn chicks 48 hour before inoculation with 5 x 10<sup>4</sup> *E. tenella* oocyst of PR or H strain. They found that weight gain and survival time of chicks was increased with reduced faecal excretion of oocysts and diminished caecal pathology.

Niculescu et al. (1971) used coccidiostats in the prervention of poultry coccidiosis. Groups of chickens were experimentally infected with Eimeria tenella or Eimeria maxima oocysts, or with mixture of Eimeria tenella, Eimeria maxima, Eimeria acervulina and Eimeria necatrix oocysts. Apart from an untreated control group, these chickens received in feed, both before and after infection, either dinitolmide, amprolium or amprolium plus. It was shown that amprolium or amprolium plus protected against Eimeria tenella and Eimeria maxima,

while denitolmide was most effective against coccidia of small intestine. It was suggested that two different types of coccidiostats should be used for treatment of fowls for coccidiosis.

In a survey of 1145 farms, Jeffers (1974b) reported that 1052 (92%) were infected with coccidiosis, and from 29 per cent of these *Eimeria tenella* was isolated. The prevalence of this organism was high in southern and south central regions of the USA. The infection rate was high in blocks where nihydrazone or dinitolmide was used in flocks treated with amprolium plus ethopabate, buquinolate and dequinolate, clopidol or decoquinate. Reciproced cross-resistance to buquinolate and decoquinate was observed in quinoline-resistance isolates.

In an another study Soltys and Wajton (1974) reported drug resistance of Eimeria tenella the against some anticoccidials. A strain of Eimeria tenella isolated from an area where coccidiostatic drugs had not been used was passaged in 2 week old SPF chicks. The chicks were treated with the following anticoccidials: coccidot (produced by the Palaway Institute), Amprol plus Veterinary (Amprolium plus "anticoccid' (amprolium) ethopabate), and 'sulfatyl' (Sulfachlorpyrazine). Out of these only two drugs i.e. coccidot and amprol plus, did not induce resistance within 20-23 passages.

McLoughlin and Chute (1974) reported that infections resulting from administration of a single oocysts isolated from an amprolium resistance strain of *Eimeria tenella* were

established in 5 of 32 chicks. Oocyst recovered from these infections were propagated in unmedicated birds and the progeny tested for resistance to amprolium. All isolates were found to be resistant. The anticoccidial index calculated for infections initiated with one isolant was lower in each of the three trials.

In an another study Jeffers (1974a) reported that crosses were made between two strains of *Eimeria tenella* differing in resistance to amprolium and decoquinate strain 91 was amprolium-resistant (AR) and decoquinate sensitive (DS), while stain 368 was amprolium sensitive (AS) and decoquinate resistant (DR) culture of the parent strains and a culture derived from a mixture of parent strain, oocysts were propagated through drug barriers to filter parasites exhibiting the respective parental phenotypes (AR/DS and AS/DR) and the recombinant phenotype (AR/DR). The ability of certain parasites in the strain cross culture to produce in the presence of the drug barriers showed that they were recumbinant phenotype (AR/DR), which had been produced through cross fertilization of gametes of the two parent strains of *Eimeria tenella*.

Medhat et al. (1974) studied histopathological examination of bursa fabrici as a mean for the evaluation of coccidiostats tested against Eimeria tenella. Experimently infected chicks with Eimeria tenella were treated with amprolium or sulphaquinoxaline in drinking water for 6 days. Histopathological examination revealed that the organisms developed in the bursa of untreated infected birds; schizonts

were seen on day 6 and 7, gametes on day 7 to 9 and oocysts on day 8 to 9 after infection. No organism was detected in the bursa of treated birds.

In an attempt to immunise chicks against caecal coccidiosis with the use of anticoccidial agent, Huang and Lee (1975) experimentally infected chicks with 20,000 sporulated oocyst of Eimeria tenella, which caused a 50 per cent mortality and a 24 per cent decrease in mean weight gain in untreated controls. Groups of chicks were then given amprolium (0.002 or 0.004%) or furozolidene (0.003 or 0.006%) in their food for following eight days, or sulfadimethoxine (0.05 or 0.1%), sulfamonomethoxine (0.05 or 0.1%), sulfadiazine (0.1 or 0.2%), sulfathiazole (0.1 or 0.2%), or sulfaquinoxaline (0.025 or 0.05%) for four consecutive days starting two days after oocyst administration. Challenge infection was given with 50,000 sporulated oocyst of Eimeria tenella 12 or 25 days after the first oocyst administration showed the best immunity occurred after amprolium, sulfadimethoxine and sulfamonomethoxine treatments. In general, immunity on the 35th day was greater than that on the 12th day. In birds challenged on the 12th day, amprolium was more effective than sulfadimethoxine, but in birds challenged on the 25th day the reverse trend was observed.

Chapman (1976) studied on the resistance of field isolates of *Eimeria tenella* species to anticoccidial drugs. He found that monensin was able to suppress oocyst number in faeces. He also reported *Eimeria tenella* was not controlled by zoalene, clopidol or monensin but was controlled by

amprolium, sulphaquinoxaline, methylbenzoquale and robonidine whereas *Eimeria acervulina* was controlled only by monensin. He found that *Eimeria* species had developed resistance to most of the anticoccidials.

In an experimental study, Bajwa and Gill (1977b) assessed the activity of amprolium against caecal coccidiasis (Eimeria tenella), and the effect of medication on the development of immunity. Activity of amprolium at the recommended level (0.024%) in drinking water was determined against infections initiated by 1000, 5000, 25,000, 50,000 and 100,000 oocyst of Eimeria tenella. Treatment was started at 96 hour after infection and was continued for ten days. The survivors were challenged with 100,000 oocysts of Eimeria tenella on the 15th day of infection to evaluate immunity. The criteria kept in view for assessing the protection afforded by the medication against the initial infection and of the resulting immunity against the challenge were growth rate, mortality, faecal and lesion scores, and total oocyst production. Amprolium provided full protection against infection initiated by 1000 and 5000 oocyst and the protective effect increased progressively with the increase in level of infection. Further, the medication caused some interference in the development of immunity to infection induced by smaller number of oocysts (1000 and 5000) but not by larger number.

McLoughlin and Chute (1975) reported the effect of sequential use of coccidiostats on development by *Eimeria tenella* of resistance to amprolium, nicarbazin, unistat, and zoalene. *Eimeria tenella* was propagated serially in group of

chickens fed with mash containing unistat (Sulphanitran), amprolium, nicarbazin or zoalene (dinitolmide) on a rotation basis. The strain of *Eimeria tenella* was propagated through 40 group of chickens and was therefore intermittently exposed 10 times to each coccidiostat (for the first five at half the recommended dose and for the last five at the recommended dose). The strain developed resistance to all the drugs except nicarbazin, but this drug showed only poor performance against infections initiated with the strain after 20 and 25 passages.

Sherkov (1977) studied the problem of the controlling coccidiosis on poultry farms in Bulgaria and described the pathological changes in chickens experimentally infected with *Emeria tenella*. He also observed the antagonism between amprolium and the thiamin which is used as feed additive.

In an another study Singh and Hussain (1978) evaluated the therapeutic activity of amprolium and codrinal against *Eimeria tenella* infection and effect of medication on the development of immunity in chicks. The activity of amprolium and codrinal against infections with 5,000, 10,000, 25,000 and 50,000 *Eimeria tenella* oocyst per chick was investigated. Amprolium provided better protection (performance index: 241, 180, 130 and 105) than codrinal (performance index, 197, 138 and 80) against all the levels of infection tested. Codrinal gave no protection against infection with 50,000 oocysts per chicks. Though both the drug interfered to some extent with the development of immunity, the immunity produced with all infection levels was still sufficient to withstand the challenge

infection. Relatively amprolium interfered more (1-12%) than codrinal (0-8%) and the interference was more marked at lower levels.

an experimental study on the development of resistance to amprolium, clopidol and methyl benzoquate, Chapman (1978) observed that resistance to amprolium and clopidol developed more readily in experiments where a large number of coccidia were exposed to the drug, either by increasing the number of oocysts in the inoculums or by increasing the number of birds in the group. When 45 birds were given two million oocysts, resistance to amprolium and clopidol appeared after 6 and 7 passages, respectively. Under similar conditions, resistance to robenidine developed after 6 passages suggesting little difference between these three drugs. Resistance to amprolium and clopidol rose gradually as the concentration of drug was increased, but resistance to methylbenzoquate appeared in a single step from sensitivity to high-level resistance. Both amprolium and clopidol-resistant lines showed on eight fold reduction in drug sensitivity. Attempts to measure the degree of resistance by calculation of the ED<sub>50</sub> were unsuccessful.

In a similar study Bajwa and Gill (1977a) observed the activity of amprolium and decoquinate against *Eimeria tenella* and the effect of medication on the development of immunity in chicken. Chicken were infected with 10<sup>3</sup>, 5 x 10<sup>3</sup>, 25 x 10<sup>3</sup> or 10<sup>5</sup> oocyst of *Eimeria tenella* and amprolium at 0.0125 per cent or decoquinate at 0.004 per cent in feed were tested against each level of infection, within 24 hours, before, 24

hours after or 96 hours after infection, continuously for 14 days. Surviving chicks were challenged with 10<sup>5</sup> oocyst after 14 days of infection. Amprolium found to be highly effective against low level of infection. Development of immunity was interfered by prophylactic medication, however, the degree immunity was inversely proportional to the oocyst dose. Therapeutic medication interfered with development of immunity resulting from low level infections only. Prophylactic 24 hours before or after infection completely inhibited the infection and the chicks remained susceptible to reinfection. Therapeutic medication after 96 hours gave little anticoccidial activity and the chicks become solidly immune to challenge. Amprolium is considered to be suitable for use in replacement flocks and decoquinate for use in broilers.

McLoughlin and Chute (1979) reported the loss of amprolium resistance in *Eimeria tenella* by a mixture of sensitive and resistant strains. A mixture of amprolium-resistant and amprolium sensitive oocysts of *Eimeria tenella* was propagated in groups of unmedicated chicks, with additional sensitive oocysts introduced in each passage. After 10 passage the response of the mixed strain to 125 ppm amprolium was approximately the same as that of the sensitive strain. Undiluted resistant strains simultaneously passed under similar conditions retained its tolerance for amprolium. These results are complemented by those of the *in vitro* phase of the studies in which the resistant strain was manually diluted with the sensitive strain.

In an experimental study, Mogollon and Molina (1977) observed the sensitivity of a field strain of Eimeria tenella to three anticoccidials. They found that the symptom of coccidiosis was not seen and non of the birds died of coccidiosis in groups of chicken treated with clopidol 0.0125 per cent or with monension 0.0121 per cent whereas the chickens treated with amprolium or Ethopabate 0.0125 per cent showed high excretion of oocyst and loss of weight and they were not protected against the infection. They concluded that the strain was resistant to amprolium.

In an experimental study, Mathis and McDougald (1981) studied the development of resistance to amprolium or dinitolmide in Eimeria acervulina and its effect on inhibition of sporulatin of oocysts. Groups of ten 7 day old chicks were fed unmedicated ration or ration medicated with amprolium (125) ppm) or dinitolmide (125 ppm). Each chicks were infected with 1500,000 oocysts of Eimeria acervulina and oocysts paned 6 and 7 days latter were allowed to sporulate for 48 hours and than used to inoculate the next group of birds. The development of drug resistance in Eimeria was determined by comparing infection produced by the first and fifth passage of oocysts. The passage of Eimeria acervulina has significantly lower sensitivity to either amprolium or dinitolmide. The lesion scores in the passage through medicated chicks were equal to those from unmedicated infected controls, and all weight gains of the infected groups were significantly lower than those of the unmedicated controls.

In a laboratory study Mathis and Mc Dougald (1982), observed that out of 41 isolates were tested for sensitivity to eight commercial amticoccidial drugs, most of the isolates were resistant to some of drugs judged by the parameters of weight gain, lesion score reduction, and a sensitivity index score. The frequency of resistance was high to clopidol, amprolium, ethopabate, nequinate, zoalene, and sulfaquinexaline. A very small amount of the isolates tested were resistant to nicarbazine, robenidine, or halofuginore.

Chapman (1984) in an another experimental study reported that a line of *Eimeria tenella* (H) resistant to robenidine, decoquinate and amprolium was produced by genetic recombination. It was not possible to obtain a cross between this line and lines resistant to clopidol or asprinocid and halofuginone. Recombination between lines resistant to asprinoid and halofuginone resulted in a loss of pathogenicity.

In a histopathological evaluation of anticoccidial activity of toltrazuril and amprolium in experimental *Eimeria tenella* infection in chicken, Lakkundi *et al.* (2002b) found that toltrozuril present the establishment of caecal coccidiasis by degeneration and disintegration of the first generation schizonts. However, in amprolium treated birds, few intact first and second generation schizonts and also micro and macrogametocytes were noticed in microscopic examination. Occasionally, oocysts were also found. Based on these studies, they concluded that toltrazuril has a coccidiocidal effect, whereas amproilium is coccidiostatic in nature.

Jin et al. (2003) reported the resistance of two Eimeria tenella isolates to five anticoccidial drugs. They studied the resistance of two Eimeria tenella isolates namely, Nanjing isolate and Fengyang isolate, to diclazuril, maduramicin, amprolium. Based the robenidine on and clopidol, anticoccidial index, relative oocyst production, per cent of optimum anticoccidial activity and reduction of lesion scores they observed that, there two isolates were slightly resistant to amprolium, sensitive or partially resistant to diclazuril, moderately resistant to clopidol, and couplet, but highly resistant to maduramicin and robenidine. It is suggested that diclazuril and amprolium can be used in Fengyang isolate effectively, clopidol should be used prudently, maduramicin and robenidine should not be used at present.

Performance index based evaluation of anticoccidials against field strains of *Eimeria tenella* in broiler chicken reported by Rana and Tikram (2001a). The efficacy of amprolium, clopidol, nitrofurazone + ferozolidone and sodium sulphadimethyl pyrimidine against four field strains of *Eimeria tenella* isolated from severe out breaks of coccidiosis of different broiler farms, at Hisar and Jind districts in Haryana state, were evaluated. Five groups of day-old chicks (n = 10) of either sex were challenged with 1 x 10<sup>5</sup> sporulated oocyst of *Eimeria tenella* at three weeks of age. The drugs were administered to chicks in four of the infected groups 24 hours prior to challenge in order to ascertain the prophylactic activity of these drug. Medication was continued up to seven days post challenge. The comparative efficacy of the anticoccidials were

evaluated on the basis of performance index based on ratio of mean weight gain, survival period and faecal score of chickens. Drug efficacy against field strains from the Hisar district, in decreasing order, was sodium sulphadimethylpyridimidine followed by amprolium, clopidol and nitrofurazone + furazolidone. For isolates from the Jind district, clopidol was most effective, followed by amprolium, sodium sulphadimethylpyridimidine and nitrofurazone to furazolidone.

Rana and Tikaram (2001b) made an in-vivo assessment of drug resistance in field isolate of Eimeria tenella. Four field isolates of Eimeria tenella designated H1-1, H1-2, J1-1 and J1-2 isolated from severe out breaks of caecal coccidiosis, were assessed for development of resistance to anticoccidials such as amprolium, clopidol, nitrofurazone + furazolidene and sulphadimethylpyrimidine. sodium The drug were administered to chicks 24 hour prior to oral challenge with 1 x 10<sup>5</sup> sporulated oocyst at 3 weeks of age. Infected untreated control and uninfected untreated control were also maintained simultaneously. A relative anticoccidial index of each isolates of Eimeria tenella against an anticoccidial determined to ascertain the relative drug resistance of coccidia. It was compared with another criteria of drug resistance, such as there was at least 50 percent difference in average droppingpan score of chicks in infected medicated control and infected unmedicated control. These criteria correlated in establishing resistance of all the 4 isolates to nitrofurazone + furazolidone, of the four isolates to clopidol and sodium some

sulphadimethylpyrimidine and none of the isolates to amprolium.

Hu-Jing Hui et al. (2000) reported anticoccidials interfere with development of protective immunity against coccidiosis in anticoccidials drugs (monensin, narasin, Eight broiler. lasalocid, salinomycin, nicarbozin, halofuginone, rebenidine and amprolium) were evaluated to the impact on protective immunity against coccidiosis in chickens after inoculation with isolates of Eimeria acervulina, Eimeria maxima or Eimeria tenella. The isolates were partially sensitive to drug, as shown by inoculation of birds with 500,000 oocyst of Eimeria acervulina and 50,000 oocyst of Eimeria maxima. When birds were inoculated with 1000 or 10000 oocysts of Eimeria maxima or Eimeria acervulina oocyst shedding was not reduced by some treatments and only about 40 percent by others, suggesting that drugs would probably not interfere with immunity. To measure the effects of drug on actual protection from reinfection, chicks were exposed to Eimeria maxima and Eimeria tenella at seventh and 14th day of age, then challenged at 25th day of age. Degree of immune protection was determined from caecal and intentional lesion scores and weight gains six days post inoculation. All medicated groups had significant protective immunity against Eimeria tenella and Eimeria maxima. These results suggest that any of the drug tested could be used without interfering appreciably with protective immunity.

In China Kong et al. (1994) observed that the 80 per cent of isolates were resistant to monensin, 66.6 per cent to

amprolium (125 mg/kg) and 53.3 per cent to clopidol (125 mg/kg) and 53.3 per cent to robenidine (33 mg/kg) but non of them were resistant to diclozuril (1mg/kg). They also found that the degree of drug resistance strength to different anticoccidials occurred in different isolates was different and most of the 15 isolates showed multiple drug resistance to two or more anticoccidials.

Sasmal and Sinha (1989) conducted on histopathological evaluation of anticoccidial activity of lasalocid and amprolium against Eimeria tenella infection in chicken. The effect of timing of medication with lasalocid and amprolium was evaluated histopathologically against experimental Eimeria tenella (5 x 103) infection in four week old male chicks to find out the endogenous stages of the parasite effected by the drugs and their effect on development of immunity. The birds medicated at zero day or one day post infection with lasalocid showed vascular degeneration and arrested development of sporozoites, whereas similar group of amprolium medicated birds showed the development of first generation shizonts. The birds treated on 2<sup>nd</sup> day post infection with lasalocid showed degeneration of first generation schizents and in subsequently treated birds no action of the drug was noticed. Groups of bird given amprolium medication showed well developed colonies of 2<sup>nd</sup> generation schizonts with well developed gametocytes on day 14 post infection all birds were challenged with  $5 \times 10^4$ oocysts. Chicks from the early lasalocid treatment groups showed development of first and 2nd generation of schizonts without colonization while the amprolium medicated groups

had only arrested development of sporozoites. In the delayed treatment groups of both medications there was no development of endogenous stages. It was cancelled that the development of immunity was partially interfered with in the easily treatment groups of both the drugs; the interference was greater in lasalocid than amprolium medication. The delayed treatment groups showed histopathologically no difference in development of immunity. When the treatment was initiated simultaneously on day 1 post infection the immunity developed was protective for both the drugs, with lasalocid appearing to be the more useful drug.

Thyagarajan et al. (1989) studied the relative performance of broiler chicks subjected to experimental coccidiosis (50,000 oocyst of Eimeria tenella) and treated with different anticoccidiosis. The following six treatments were given: 0.16 percent sufadimidine in drinking water (IMS); 0.22 percent ptoluene-sulfonyl betamethoxyethyl-urethane (IMT): 0.01 (nitrofural) (IMN); nitrofurazone 0.24 percent amprolium hydrochloride (&MA); infected and unmedicated control (IUM); uninfected and unmedicated control (UNUM). Body weight gain, and feed and water intake significantly reduced in all infected groups. Post-infection mortality due to coccidiosis was highest in IMN and IMI (82.5 percent on day 10) groups. Mortality in unmedicated control was 65.0 per cent, whereas in the IMS and IMA groups it was 52.5 and 50 per cent respectively.

Mathis et al. (2004) studied on the dtermination of appropriate time to administer toltrazuril for the control of

coccidiosis in broiler chickens. They observed that the use of toltrazuril for two consecutive days in the drinking water between days 10 and 14 would be the best time for good.

#### Sulphachloropyrazine:

Since its introduction in 1967, the drug has been extensively used in the treatment of poultry coccidiosis.

A comparative study on the therapeutic affect of E and b<sub>3</sub> (sulphachlorapyrazin) and Abi-Zet50 against ceacal coccidiosis in white leghorn chicks, conducted by Manuel et al. (1978). They infected chicks aged 21 days with 65,000 oocysts of Eimeria tenella and treatment started after 24, 28, 72 or 96 hours of infection with Esb<sub>3</sub> (Containing 30 per cent sulphachloropyrazine; (6-chloro-zpyrazyl)- sulphanilamide) at dose of 1.5g/litre drinking water or with Abi-zet<sub>50</sub> (containing sulphadimidine 50 per cent plus pyrimethamine) at dose of 0.5g/litre of drinking water. Neither of these drugs prevented destruction of the caecal wall by the parasite, but birds treated within 24 or 48 hours showed the least caecal damage. When treatment started at 24 hours or 48 hours post infection, both the drugs were equal in their anticoccidial efficacy, but when the treatment started at 72 hours post infection, Esb<sub>3</sub> found to be more effective. Birds treated with Esb<sub>3</sub> consumed more feed, drank more water and gained more weight than those treated with Abi-zet 50.

In an another experimental study, Penev and Lozanov (1983) reported the action of sulphachloropyrazine sodium (Esb<sub>3</sub>) on endogenous development of *Eimeria tenella*.

Sulphachloropyrazine was given in drinking water of 84 ten-day-old chicks for three successive days, at a dose of 1.5 g/litre, commencing at the same time as infection with 50,000 sporulated oocysts of *Eimeria tenella*, or 1-7 days after infection. The organism found to be most sensitive to the drug 2-3 days after infection; no effect was observed on development of coccidian parasite when given at the time of infection or 24 hours post infection. When given 4 to 7 days after infection, the drug inhibited the development of the coccidia, but did not produce degenerative changes in them.

Rahman et al. (1993) evaluated the efficacy of sulphachloropyrazine (Esb<sub>3</sub>). They gave Esb3 in drinking water to 950 birds out of the 1000 birds at a dose of 1 g/litre, on a private farm in which coccidiosis (Eimeria acervulina and Eimeria tenella has been diagnosed as the cause of diarrhea and mortality. They observed that diarrhea was controlled within 24 hour and oocyst production by three days. After seven weeks, feed conversion and body weight gain were considerably higher than in the 50 non-treated control birds. No adverse effect of the drug were seen on the birds.

Samad and Chakraborty (1993) studied the chemotherapeutic management of acute out breaks of caecal coccidiosis in broiler birds. A group of 1500 birds out of 2408 broiler chicken were treated with sulfaclozine sodium (ESb<sub>3</sub>) at a dose of 1g/litre in drinking water for 3 days and another group of 908 birds was treated with sulfaquinoxaline (Embazin) at dose of 1.5g/litre drinking water for 2 to 3 days. They found Embazin was more effective in treatment and

reduced mortility in the group from 9.0 to 0.9 per cent compared with Esb<sub>3</sub> with reduced mortality from 5.7 to 2.3 per cent.

In an another trial Padmavati et al. (1995) evaluated the therapeutic efficacy of Esb<sub>3</sub> against experimental mixed coccidial infection (Eimeria tenella, Eimeria necatrix, Eimeria maxima and Eimeria acervulina) in chicken. A total 150, two week-old chickens divided into three groups, each with 50 birds. The drug was administered at a dose rate of 1.0g/litre drinking water on day 2, 3 and 4 day post infection in group I and on days 2,4,6 and 8 post infection in group II, chicks in group III kept as untreated controls. Ten birds from each group were autopsied on day 5, 7 and 10 post infection. Average faecal and lesion scores in both group of treated birds were both reduced to 0.5, compared to 3.0 and 3.5 respectively in group III. The weight of birds on group I and II averaged 148.3 and 152.0g, respectively, compare with 119.5g in group III. There was no mortality in the treated groups. The drug was well tolerated and the authors did not observe any apparent signs of toxicity.

Panda et al. (1999) evaluated the efficacy of Esb<sub>3</sub> in the treatment of experimental Eimeria tenella infection in broiler chickens. Sulphachloropyrazine was added at a dose rate of 1.5g/litre to the drinking water of a group of three-week-old broiler chicks when they showed clinical signs of coccidiosis three days after oral inection with sporulated oocysts of a laboratory culture of Eimeria tenella. Compared with an infected, unmedicated group of birds, it was observed that the

drug was effective in checking both morbidity and mortality the treated birds showed a weight gain of 98.38 per cent (100% in uninfected, untreated birds), better feed conversion, very low faecal scores, a very low oocyst output, a good performance index, and only mild damage to the caecal mucosa.

Hoang et al. (1999) reported the recent observations on coccidiosis in industrial poultry in the vicinity Hochi minh city. The prevalence of coccidiosis in industrial poultry was examined during 1996 to 1998. The parasites showed resistance to Rigecoccin and Amprolium but were sensitive (96.98 per cent) to newly introduced drug such as Baycox, Sacox and Esb<sub>3</sub>.

Siddiki et al. (2000) reported sulphonamide resistance in field isolates of chicken coccidian in Bangladesh. The prolonged and indiscriminate use of sulphonamides in the treatment of chicken coccidiosis has resulted in the inevitable emergence of drug resistant strain of Eimeria spp. They found that Eimeria spp. had developed a significant level of resistance against the drug, the degree of which however, varied between the isolates. The increased dose rate may not be an alternative to the existing drug resistance. This may cause toxic effects to the birds which may also be harmful to humans.

The drug Esb<sub>3</sub> is found to have identical effect on coccidiosis parasites of other avian species. Dubovoi (1976) studied on therapy and Chemotherapy of coccidiosis in Turkeys. Out of nine coccidiostats tested, sulphadimethoxine 0.1 per cent solution replacing drinking water for 10 days gave

the best results. Esb<sub>3</sub>, sulphadimezine (Sulphadimidine), trichopol (metronidazole) and Rijecocein (Clopidol) used at the recommended schedules were also effective. The therapeutic efficacy of sulphodimethoxine was estimated at 85.7 per cent and treatment was economically effective, two cources of 5 days with an intervals of 3 to 5 days are recommended.

Yuzev (1980) studied on the pathogenicity, differential diagnosis, prophylaxis and therapy of cocciddiosis in pheasants with particular reference to Bulgaria where disease in caused by Eimeria colchici, Emeria duodenalis, Eimeria phasiani, Eimeria pacifica and Eimeria megalostoma. Esb<sub>3</sub> (1g/1 litre of water), Pancoxin (567g of premix/100kg feed), DOT (30g/10 litre of water), furazolidone (0.07% of feed) and sulphadimezine (4.4g/kg feed) are successfully used on industrial farms.

Koinarski and Sherkov (1987a) tested various coccidiostats against experimental *Eimeria adenoeides* infection in turkeys. Robenidin, Esb<sub>3</sub> (Sulphaclozine), the Bulgerian preparation trimidin and toltrazenil were among the most effective drugs. They observed that the efficacy was dependent upon the timing of treatment.

Koinorski and Sherkov (1987b) studied on the comparison of some anticoccidials for treating *Eimeria adenocides* in turkeys. Twelve groups each of ten 18-day old poults were infected with 90,000 sporulated oocysts of *Eimeria adenocides*. They were treated with one of four anticoccidials added to the drinking water for various periods, between one hours and seven days after infection. The best results were

obtained with sulphachlorpyridazine sodium (Esb<sub>3</sub>) at 1.5 g/litre and Trimidin (manufactured in Bulgoria) at 5ml. per litre. The sulfonamide preparation 'Coccistop' (Intervet, Netherland) and coccibio (IFFA-Mexiuex, France) were slightly less effective.

#### Toltrazuril:

Toltrazuril is one of the few anticoccidial compounds introduced recently. Though the drug was used in the treatment of cocciodiosis extensively after its introduction in 1985, the report on the development of the resistant against the drugs was meager.

Varga and Csiko (1973) studied on the anticoccidial activity of toltrazuril in chickens. They observed coccidiostatic effect of toltrazaril on first schizogony and coccidiocidal effect on second schizojony.

In a similar study Papparella *et al.* (1985) studied the efficacy of toltrazuril in broiler chicken. They observed that chicks treated with toltrazuril at a dose rate of 100 ppm. showed better feed utilization and the lowest oocyst excretion than the group treated with monensin.

Merlanti and Quaglio (1986) studied the therapeutic efficacy of toltrazuril in comparison with sulphechlorpyrazine against the coccidiosis caused by Eimeria tenella, Eimeria acervulina, Eimeria necatrix, and Eimeria maxima. The effects of two drugs was assessed, based on average daily weight gain, feed conversion, and number of lesions. Their effects in respect of weight gains and feed conversion were comparable, except that toltrazuril gave better results with Eimeria necatrix.

Moreover, unlike the results of sulphachlorpyrazin treatment, no oocysts were recovered from the faeces of chicks treated with it.

In a histopathological evaluation of anticoccidial activity of toltrazuril and amprolium in *Eimeria tenella* infection in chicks. Lakkundi *et al.* (2002a) found that toltrazuril prevented the establishment of organism by degeneration and disintegration of the first generation schizonts. However, in the amprolium treated birds, few infact first and second generation schizonts and also micro and macro gamitocytes were noticed on microscopic examination. On the basis of histopathological observed they concluded that toltrazuril has a coccidiocidal effect, where as amprolium was caccodiostatic in nature.

In an another experiment Lakkundi et al. (2002b) tested the effect of toltrazuril and amprolium on body weight and feed conversion efficiency of broiler chicken experimentally infected with Eimeria tenella found that toltrazuril treated birds did not excrete oocyst, but had better body weight gain and feed efficiency than uninfected birds. The caecal lesion score was mild in this group. In the amprolium treated birds, the OPG was less than in infected controls and mean body weight was lower than toltrazuril treated birds. However, cumulative feed efficacy was similar in both treatments. In a histopathological evaluation of anticoccidial activity of toltrazuril and amprolium in experimental Eimeria tenella infection in chicken.

In a floor pen study Mathis et al. (2003) compared the anticoccidial efficacy of tolrazuril as a stand alone treatment and as an additional treatment with infeed anticoccidial

programmes. Toltrazuril was administered on days 18 and 19 post infection in the drinking water at the dose rate of 7 mg/kg of body weight. The birds were also treated with nicarbozine (125 ppm on days 0-14), and with salinomycin at the dose rate ot 66 ppm on days 15 to 35 along with previously administered toltrazuril. The controls were normedicated non infected and infected non-medicated. On day 14, the birds (except non infected) were exposed to coccidial oocysts (Eimeria. acervulina, Eimeria. maxima and Eimeria. tenella) seeded litter. On days 21, 28, 35 and 42, birds and feed were weighed, four birds per pen were sacrificed for lesion score and litter oocyst counts were performed. They found that coccidiosis was moderately controlled in the anticoccidial treated birds without tolrazuril. Performance in the birds treated with tolrazuril plus was equal to or were even better (P<0.05) than the binds treated without toltrazuril. Toltrazuril was equal to the non infected birds in performance. Toltrazuril completely eliminated all coccidial lesions and dramatically reduced oocyst shedding. The performance data, lesion scores and oocyst counts showed that a two days treatment with toltrazuril successfully controlled the coccidiosis with no relapse of infection.

Grief (2000) hypothised that chicken immune sera after toltrazuril treatment which may be involved in the enhancement of immunity to *Eimeria* spp. Tolrazuril was administered either continuously with the feed at a dose rate of 5 ppm or in drinking water at a dose rate of 25 ppm on two successive days. A mixed infection of *Eimeria*. acervulina,

Eimeria. maxima and Eimeria. tenella was conducted on day 14, followed by a similar challenge infection on day 43. The drug acted against all intracellular parasite stages from 1 to 168 hour post infection, intracellular schizonts of generation I, II and III and intracellular gamonts. Since the damaged stage remained in the host cells for a prolong period, during which they acted as antigens which were recognized by immune system. Immune sera during therapeutic treatment with toltrazuril showed a high antibody titre of 1:100 and 1:250 in IFAT after challenge. Toltrazuril did not interfere with process of parasite protein recognition by the immune system during infection. There was a tendency for a higher IgG response after treatment with tolrazuril.

Pathak and Kappor (1998) evaluated the anticoccidial efficacy of toltrazuril against Eimeria tenella infection in broiler chicks. A total of 75, 3-weeks-old broiler chicks were each infected experimentally with 50,000 oocyst of Eimeria tenella. After three days, groups of 25 chicks were given toltrazuril at a dose rate of 25 mg/kg in drinking water for two days, or amprolium at 30g/25 litres for five days; the third group of 25 chicks were kept as untreated controls. Faecal samples were examined for oocysts, from day five to day 20 post infection. Toltrazuril was 100 per cent effective from day 5, but oocysts were present from day 10 in amprolium treated group. The feed consumption and growth rate of the tolrazuril treated birds were also higher compared with other two groups.

However, Duan-JiaShu and Liu (1995) studied on the action of toltrazuril on *Eimeria tenella* and its effect on the

development of immunity in chickens to coccidiosis. No oocyst were found from days 6 to 20 post infection in chickens which had been inoculated with 2000 oocyst and treated with toltrazuril at the recommended level of 25 ppm in drinking water for two consecutive days from days zero post infection. By giving treatment on different days (1 to 5 days pi) after infection with 8 x 104 oocysts, it was found that peak activity was between day 0 and day 2 post infection. Treatment initiated on day 4 post infection had no effect on infection. The anti coccidial action persisted to some degree on day one after toltrazuril withdrawal, but almost disappeared by 2nd day. Toltrazuril did not interfere with the development of anticoccidial immunity.

In an another study Rahbari *et al.* (1996) reported the resistance development of field isolated *Eimeria* spp. against some anticoccidial drug. They found that amprolium was unable to prevent oocyst shedding where as lasalocid was efficient in reducing oocyst output to a significantly low level (P<0.05). Sulfaquinoxalindiaveridine could not inhibit the shedding of *Eimeria acervulina*, where as toltrazuril was highly effective *Eimeria maxima* and *Eimeria necatrix*.

A study to Eimeria tenella infection in broiler chicken was conducted by Ramadan et al. (1997). They found that both drugs were highly effective against Eimeria tenella. Treatment improved the body weight gain and survival percentage. Intestinal lesions, faecal and oocyst scores and oocyst shedding in droppings were significantly reduced by both drugs. However toltrazuril gave better protection then

halofuginone; when administered at a dose rate of 75 and 150 ppm for four to five days after inoculation.

In a comparative study on the efficacy of sulpha chlorpyrazine and toltrazuril Laczay et al. (1995) observed that both drugs prevented coccidiosis related mortality and decrease of weight gain to a similar degree, but toltrazuril was more effective in reducing intestinal lesions and faecal scores, when treatments were initiated 24 hour post infection in the battery study. When medication was delayed until 72 h of inoculation, the sulfonamide proved to be more effective in preventing reduction of weight gain and intestinal lesion caused by the parasites. Under simulated use conditions both drugs showed an appropriate anticoccidial efficacy without major differences between them.

In a battery trial, Muangyai et al. (1991) studied on the efficacy of toltrazuril in prophylaxis and treatment of caecal coccidiosis in broiler chicks. They found that 25 ppm and the drugs at 75 ppm concentration were most effective between 2 pree day to 2 days post infection period. The parameters used were lesion score reading, haematocrit value, oocyst per gram count, clinical signs and mortality.

In a comparative chemotherapeutic study on tolrazuril and maduramicin, Muangyai et al. (1990a) observed that subclinical coccidiosis due to Eimeria acervulina and Eimeria maxima infection occurred in the toltrazuril treated group and Eimeria acerulina, Eimeria maxima and Eimeria tenella occurred in the maduramicin treated group. They found higher

oocyst per gram of faeces in the maduramicin treated group than in toltrazuril treated group.

Muangyai et al. (1990b) studied on prophylaxis and treatment of chicken coccidiosis in replacement pullets with toltrazuril. The prophylactic efficacy of toltrazuril was investigated in 3 groups of 10-day-old replacement pullet chickens. The drug was given at an interval of eight hours at a dose of 25 and 50 ppm in drinking water to group I and II respectively, while group III was kept as infected nonmedicated control. Growth rate was not substantially different among the three groups though mortality was higher in group III than the other groups. In week 6, subclinical and clinical coccidiosis appeared in groups I and III respectively. Eimeria tenella and Eimeria mexima were recovered from group I and Eimeria tenella, Eimeria necatrix and Eimeria acevuline from group III. Lesion scores of Eimeria necatrix in group III were significantly different from the other groups. Lesion scores in group I and III were significantly different from group II. Treatment with toltrazuril was immediately applied at week 6 to chickens of groups I and III for 24 at a dose of 25 ppm and for 8h at a dose of 50 ppm, respectively.

A study to evaluate the efficacy of toltrazuril and sofra vitamine against avian coccidiosis was conducted by Deghidy and Askalany (1989). They found that treatment with both drugs were very effective, passage of oocysts in faeces was stopped 2-3 days after treatment and weight gain began nine days post infection.

In an another study Varga (1990) observed that toltrazuril can effectively control coccidiosis if the treatment is started at the time of first clinical signs, but can not be used to prevent high mortality in diseased animals with severe lesions in caeca. Hence, he suggested that early diagnosis is of great importance in the treatment of coccidiosis.

Vertommen et al. (1990) reported that when toltrazuril applied intermittently during five consecutive periods, was effective in preventing coccidiosis in a broiler flock, and its effect lasted for several weeks. However, coccidiosis began to recur during the fifth rarity period, suggesting the development of resistant coccidian. In chicks infected with a mixture of Eimerica. acervulina, Eimeria tenella and Eimeria maxima oocysts at 7, 10 or 15 days of age, toltrazuril treatment starting at 10-11 days of age protected the chicks for at least a week. One of 4 isolates of coccidian from field trial was resistant to toltrazuril.

Choi et al. (1988) reported that sulfadimethoxine and toltrazuril were effective against Eimeria acervulina in naturally infected chickens with anticoccidial indexes of 161.4 and 160.4 respectively. Sulfadimethexine, amprolium plus sulfaquinoxaline and toltrazuril were effective against natural Eimeria tenella infection, with anticoccidial indexes of 165.7, 162.5 and 60.9 respectively. Amprolium alone and furaltadone were not effective against either parasite and had anticoccidial indexes of less than 110.

In an another study Chapman (1989) reported that treatment with toltrazuril sulfaquinoxaline/pyrimethamine

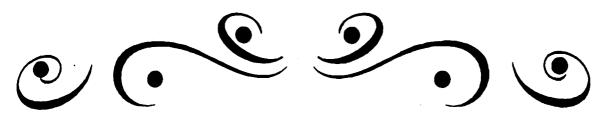
and amprolium/ethopebal prevented mortality in chickens infected previously with fied isolates of *Eimeria tenella*. Amprolium/ethopabate were highly effective in reducing lesion caused by the parasite. Few oocyst of *Eimeria tenella* were detected in birds treated with sulfaquinoxaline pyrimethamine or amprolium/ethopabate and none in those treated with toltrazuril.

Awadalla et al. (1998) studies the effectivity of some anticoccidial drugs on immunity and performance index in broiler chicken. They found that there was little interference in the development of immunity against Eimeria tenella in a group which was given toltrazunil at a dose of 1ml/litre (immunity index 296) but chicken in this group had better body live-weight gain and survival percentage. However, the group that was treated with anticoccidials at a dose of 1 gm/2 litres (immunity index 256) showed interrupted development of immunity.

However, Dillon et al. (2004) reported efficacy of toltrazuril against different level of Eimeria tenella infection in chicken. The efficacy of toltrazuril against different level (1 x 10³, 5 x 10³, 1 x 10⁴, 2.5 x 10⁴, 5 x 10⁴ and 1 x 10⁵) of Eimeria tenella infection in two weeks old chickens were evaluated. Treatment was given at 2 ml./litre of drinking water for two consecurive days, 98 hour post infection. The performance indexes recorded from lower to higher level of infection were: 266, 258, 259, 216, 197 and 135 respectively. The treatment resulted to lower mortality and reduced oocyst production. They also observed that the drug interfered in the development

of immunity only at the lower level of infection (1 x  $10^3$  and 5 x  $10^3$  oocyst /bird), but at heavy infection development of the immunity was independent of the drug administration.

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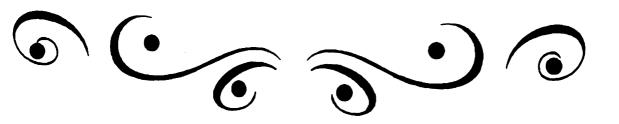




CHAPTER - III

## MATERIALS

AND
METIODS



## MATERIALS AND METHODS

The present studies were conducted to evaluate the resistane of some coccidiocidal chemical compounds such as Amprolium, sulphachloropyrazine and Toltrazuril against the clinical coccidiosis induced by the *Eimeria tenella* field isolate. The experiment was carried out at Bihar Veterinary College, Patna. The materials used and the procedures adopted during experiment are described below:

#### 1. Survey of poultry birds for incidence of coccidiosis:

A brief survey was made on the incidence of coccidiosis in poultry bird in flock of local poultry farms falling under Desari block of Vaishali district. Survey was also conducted by post mortem examination of died birds in the local poultry farms and also on the birds slaughtered in local retail shops. The faecal samples and intestinal scrapings were screened for the presence of Eimeria oocyst and their morphological parameters were also studied to assess the incidence of Eimeria spp. in poultry birds. This was also supported by the lesions during the post-mortem examination of suspected birds. The species identification of the Eimeria spp. was made on the basis of morphological characteristics of sporulated oocyst, sporulation period and intestinal lesions.

### 2. Isolation and preparation of the infective inocula:

In order to isolate the oocysts of Eimeria tenella, coccidial lesions were noted in caeca and ceacal content collected form dead birds. These contents were cultured in freshly prepared

2.5 per cent potassium dichromate solution kept in wide Petri dishes and incubated at 21°C in a BOD incubator, as described by Richardson and Kendall (1963).

The culture media was generated twice daily and water was added upto the mark to compensate the fluid loss through evaporation whenever necessary. The samples were examined daily microscopically to observe the development of the oocyst and sporulation time was noted. After the completion of sporulation the suspension was pooled, centrifuged at 1500 rpm, supernatant discarded and the sediment containing sporulated oocyst was washed 5-6 times by adding distilled water and repeating the process in order to remove the salt of potassium dichromate. Then the sporulated oocysts were concentrated by salt floatation technique. Such concentrated oocyst were again washed with distilled water by repeated dilution and centrifugation for 3-4 times and finally preserved in the refrigerator at 4°C.

#### 3. Procurement and maintenance of experimental chicks:

To start the experiment, 350day - old cockerel chicks were purchased from Patna and were reared in deep litter system. Before the start of the experiment, the brooding houses were cleaned, washed and disinfected. About 5 cm depth of fresh, dried saw dust was used as litter material. Proper management of litter was followed during experiment. Each group of birds were kept in separate compartment of brooding house. Each brooder was provided with four bulbs of 100 watt for lighting and maintaining the temperature. A

temperature of 95 °F was maintained for first week, then it was reduced by 5 °F per week till it riched to 7 °F.

Birds of all experimental groups were reared under similar managemental and housing conditions. All possible measures were adopted to prevent containination of drinking water, feed, litter with extraneous coccidial oocyst during entire study period.

The birds of all groups were provided with adlibitum feed and water for entire period of the experiment, except for few hours prior to the inoculation of oocyst to chicks. Usually chickens consume about 2 to 2.5 ml. of drinking water for each gram of feed consumed during the starting and growing period. The chick ration were prepared as per the formula given in table–1 under the investigators own supervision to ensure that these do not contain any anticoccidial agent. The ration for chicks was formulated (Table-1) as per the specifications given by Panda (1989).

Table - 1: Composition of ration used for experimental chicks:

Feed Ingredients	Quantity (Percentage)
Maize	43.75
Rice polish	10
Groundnut cake	14
Fish meal	10
Blood meal	3.5
Animal fat	3.00
Bone meat	0.15
Common salt	0.50
Minerals and vitamins	0.10
	100%

## 4. Isolation of single line oocyst and preparation of donor chicks:

Agar media was used to isolate single oocyst of drug resistant strain of *Eimeria tenella*. The agar media was prepared from two per cent aquous sterilized solution of Agar after pouring the same on sterilized glass Petri dishes to make a thin layer. These Petri dishes containing agar solution were refrigerated for solidification and stored at 4°C temperature till further use.

The fluid containing sporulated oocysts were first diluted in distilled water and then spread over a piece of agar media placed on a glass slide. The single oocyst was identified with the help of microscope and directly fed to one-week-old chicks. A total of five chicks were infected at the age of one week and reared under strict hygienic conditions in individual cages. The birds were kept on sterilized feed and water to check any extraneous coccidial infection. Following introduction of the infection, faecal droppings of the chicks were screened daily.

All the faecal oocysts were separated and kept in 2.5 per cent potassium dichromate solution at room temperature for sporulation. The infected and surviving chicks were sacrificed and the faecal contents were collected. These materials were mixed thoroughly and kept in 2.5 per cent potassium dichromate at room temperature for sporulation. These sporulated oocysts were washed, concentrated and stored at  $4^{\circ}$ C.

The sporulated oocysts were counted by Mc Master technique as per the procedure described by Shashikala (2005).

#### 5. Confirmation of the species of Eimeria tenella:

The confirmation of the species, *Eimeria tenella* and their prevalence was done with the help of the morphological characteristics of sporulated oocysts, sporulation period, prepatent period, intestinal lesions, pathogenicity and clinical manifestation of the disease.

#### 6. Experimental design:

The experiment was conducted with 350 day old cockerel chicks purchased from central poultry breeding form, Patna. After receiving chicks from hatcherie, they were randomly divided into seven different groups having 50 chicks in each group.

All the chicks were kept under coccidia free condition and all the possible measures were adopted to keep the flock healthy. The chicks of all groups were reared in deep litter system in individual compartment and the groups were designated as group I, II, III, IV, V, VI and VII.

Anticoccidial compounds, like, Amprolium, Sulphachloropyrazine which have been used to treat the outbreak of coccidiosis since last several years in addition to newer molecule, Toltrazuril were selected for the trials.

In order to evaluate the efficacy of these anticoccidiocidal agents, the chicks of each groups were kept in separate room and were maintained under similar conditions. From day one onward the chicks of all group were fed anticoccidial free

Table -2: Chemistry of the anticoccidial used in the trial.

Pharmacologic name	Amprolium	Sulphachloropyrazine	Toltrazuril
Classifying group		Sulphonamide	Symmetrical triazone
Chemical composition	Amprolium Hydrochloride	Sulphachloropyrazin sodium	Toltrazuril
Chemical structure	H <sub>3</sub> C N <sub>2</sub> N <sub>3</sub> C N	Na CI · HZO	
Trade name	Amprolium	ESb <sub>3</sub>	Comatril
Activity	Coccidiocidal	Coccidiocidal	Coccidiocidal
	Wockhardt limited		Ranbaxy Animal Health
Manufacturer	Wockhardt towers  Bandra, Kurla Complex.	Animal Health Sector	A-3, Okhla Industrial Aria,
	Mumbai-400051	Mumbai-400020	Phase-1, New Deini-110020

ration. The experimental trial was conducted as per schedule given in table – 3.

Table - 3 : Experimental Design

Group No.	No. of birds group	Oocyst inoculums/ chick	Anticoccidial schedule	Assessment of drug efficacy
I	50	20,000 Oocyst on day 14	Amprolium – 0.024% in drinking water 4 <sup>th</sup> day Post Infection (PI) for 2 weeks period.	Weight gain, O.P.G.per cent mortality, lesion score and faecal score
II Control	50 ·	Zero	Uninfected amprolium treated control	"
III	50	20,000 Oocyst on day 14	Sulphachloropyrazine sodium-0.03% in drinking water 4 <sup>th</sup> day P.I.for 3 days	"
IV Control	50	Zero	Uninfected Sulphachlorepyrazine sodium treated control	"
V	50	20,000 Oocyst on day 14	Toltrazuril 2.5, 7 mg/kg body weight in drinking water 4 <sup>th</sup> day P.I. for two consecutive day	99
VI Control	50	Zero	Uninfected Toltrazuril treated control	"
VII Control	50	20,000 Oocyst on day 14	Infected untreated control	"

On the day 14, all the birds of groups I, III, V and VII were infected with 20,000 sporulated oocyst of Eimeria tenella. On the 4th day post infection, birds of group I, III and V were different coccidiocidal compounds, like with treated amprolium, sulphachloropyrazine and toltrazuril respectively. Whereas, the birds of group II, IV and VI were maintained as uninfected treated control. The birds of group VII were kept as untreated infected control. The groupwise schedule of treatment is tabulated in table-3. On the day 7th post infection, 20 per cent of birds of each group were weighed, sacrificed and caecal contents collected and their caecal lesion were scored. On the 14th day post infection another batch of birds were again sacrificed and studied as below. The mortality rate was also observed.

Birds of each groups were examined for general appearance, gait, behaviour, body weight, feed and water and clinical symptoms like respiratory consumption symptoms, nervours symptom, G.I. symptoms etc. both in the pre and post treatment period. Abnormal constituent of faeces like presence of blood, mucles and other abnormalities were also noted. During the post treatment period the faecal samples from each group was collected randomly and examined microscopically for the presence of oocysts. Microscopic examination for the presence of oocyst in the randomly collected faecal samples from each group was done after the treatment. Efficacy of the drugs were assessed from the parameters like weight gain, O.P.G., per cent mortality, lesion score and faecal score.

#### Challenge infection:

All the surviving chickens were challenged with 40,000 sporulated viable oocyst of *Eimeria tenella* on 14<sup>th</sup> day of infection. All the parameters like lesion score, faecal oocyst count (OPG), body weight gain and mortality rate were observed. All the surviving birds were sacrificed on 7<sup>th</sup> day post challenge and lesion score was recorded.

#### Different parameters studied:

(a) Weight gain: On the basis of body weight, weight gain in birds of different groups were calculated separately. The weight gain were recorded by substracting the first weight from the last weight of birds, which were recorded on day seventh, the day of infection and on 7th and 14th day post infection. The birds were individually weighed by a single pan mechanical balance.

The ratio of mean weight gain, observed at weekly intervals, for the entire test period of the treated infected groups to the mean weight gain of treated non-infected groups was calculated on the basis of the formula suggested by Morehouse and Baron (1970) and Shumard and Callender (1970) with shight modification.

Mean weight gain of the treated infected survivors

Weight gain percentage = ×100

Mean weight gain of the treated non infected survivors

#### (b) Oocysts per gram of faeces (O.P.G.):

For this purpose, faecal samples were collected randomly from each group of birds starting from zero-day of infection, upto 8th day post infection. The faecal oocysts were isolated, concentrated and counted by Mc Master method (1977).

#### (c) Mortality rate (Mortality per cent):

To determine mortality rate chicks were observed daily for clinical manifestation and mortality after introduction of infection. Death due to other reasons was also recorded. The mortality rate was determined for each group of birds on the basis of formula given below:

#### (d) Lesion score:

Lesion scoring was done by comparing quantitatively the extent of gross lesions formed in different groups of birds used in anticoccidial trial programme. This was done on 7th and 14th day post infection and the results were expressed as 0 to +4 on the scale proposed by Johnson and Reid (1970). The average lesion score was calculated on the basis of total number of birds sacrificed on each occasion. The per cent protection in the formation of lesion was determined by using the lesions score and calculated by substracting the average lesion score of the group from the maximum expected score (4.00), dividing the resultant figure by 4 and multiplied it by 100 as described by Singh and Gill, 1976.

<b>Lesion Score</b> 0	Descriptions: No Gross lesion / normal
+ 1	Very few scattered patches on the caecal wall; no thickening of the caecal wall with normal caecal content.
+ 2	Numerous caecal lesions coupled with bloody caecal contents; mild thickening of caecal wall.
+ 3	Large amounts of blood or caecal cores present; caecal walls greatly thickened; little faecal content present in the caeca.
+ 4	Caeca greatly distended and filled up with blood or large caseous cores; faecal

#### (e) Faecal score:

To observe faecal score faecal samples were examined qualitatively and faecal scoring was done on the basis of the presence of blood and mucous in the faeces as per the method used by Morehouse and Baron (1970).

The result were scored as 0 to + 4.

debries lacking or mixed with cores. All

dead birds were scored as + 4

Faecal Score	Descriptions:
0	Normal droppings without blood
+ 1	Slight blood and mucous followed by normal consistency of faeces.
+ 2	Moderate bloody droppings with little amount of normal faeces.
+ 3	Extensive bloody droppings coupled with watery faeces.
+ 4	Extensive bloody droppings with severe haemorrhagic diarhoea.

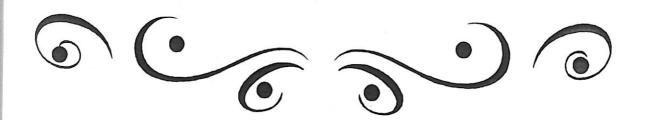
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CHAPTER - IV

# RESILIS





In the present study the results and observation made on the prevalence of coccidiosis in poultry birds in the flock of local poultry farms in blocks of Vaishali district and slaughtered in the vicinity of Hajipur. The relationship of age, sex and seasonal variation on the incidence of infection was recorded.

The results of the experimental studies conducted to evaluate the efficacy of some chemical compounds viz. Amprolium, sulphachloropyrazine and Toltrazuril on chicks are being depicted.

#### Prevalence of Poultry Coccidiosis:

Faecal samples of 508 birds were examined for the presence of coccidial oocyst. The result shows that out of 508 birds examined, 84 were found to shed coccidial oocyst in their faeces. The results were summarized in table 4 to 6.

The incidence of coccidiosis was more common in young birds (22.96%) in the age group of two to 10 weeks than the adults (9.24%). The result presented in table-4 and fig.-1 and further indicate that the young birds were significantly more susceptible to disease than the older birds. The Chi-square test also confirm highly significant effect (P<0.01) of different age group of birds on the incidence of coccidiosis.

The data on incidence of coccidiosis in different performing type of birds (Table-5, Fig.-2) revealed that the disease was more common in broilers (21.85%) than the layers

Table - 4: Incidence of coccidiosis in different age groups of chickens in and around Desari, Vaishali.

Age of birds	Total no. of birds examined	No. of infected birds	Percentage of infected birds	$\chi^2_1$ d.f.
Young (2-10 weeks)	270	62	22.96	
Adult (10 weeks onward)	238	22	9.24	19.09**
Total	508	84	16.53	

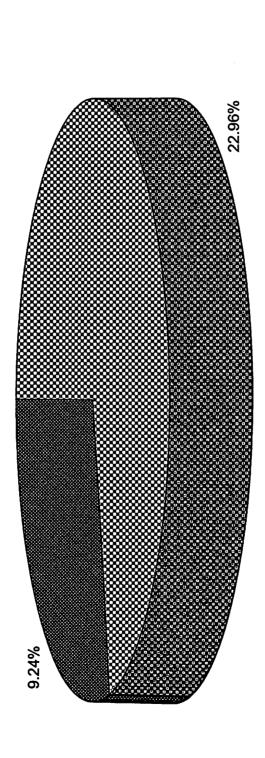
<sup>\* \*</sup> Significant at P<0.01

Table - 5: Incidence of coccidiosis in different Category of chickens in and around Desari, Vaishali.

Category of poultry birds	Total no. of birds examined	No. of infected birds	Percentage of infected birds	$\chi^2_1$ d.f.
Broilers	270	59	21.85	
Layers	238	25	10.50	11.79**
Total	508	84	16.54	

<sup>\* \*</sup> Significant at P<0.01

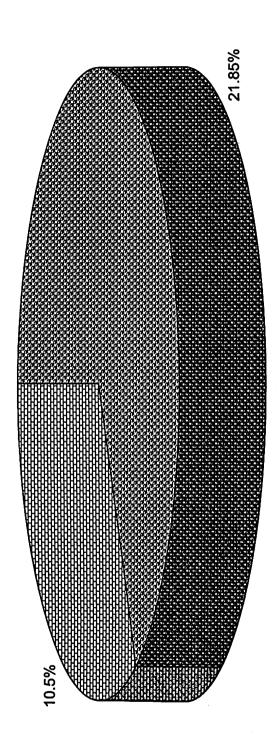
Fig. 1: Pie diagram showing incidnece of coccidiosis in different age groups of chicken in and around Hajipur.



Young weeks

Adult weeks

⊠ Broilers 🛱 Layers



(10.50%). The Chi-square test of significance reveal that the performance of the birds have significant effect (P<0.01) on the incidence of the disease.

The seasonal effect on incidence of coccidiosis is presented in Table-6. Result shows that the disease was highest in rainy season (33.84%) and lowest in winter (8.06%). The highly significant effect of season on coccidiosis in poultry was established by Chi-square test. The calculated value of X<sup>2</sup> for 3df was 38.91.

The identification of species of coccidian parasite revealed that Eimeria tenella was the most predominant species (8.46%) followed by Eimeria necatrix (2.45%), Eimeria acervulina (2.31%) and Eimeria maxima (1.57%). The occurrence of other species was occasional and constitute a meagre 1.18 per cent (Table-7.)

# Chemotherapeutic efficacy of anticoccidial agents used for trial:

To evaluate the efficacy of anticoccidial agents viz. amprolium, sulphachloropyrazine and toltrazuril, certain common parameters such as weight gain, faecal score before death and lesion score on natural death or after sacrifice as well as the rate of mortality were considered. According to the experimental design and the methodology described earlier, 350 chicks were randomly divided into seven groups each consisting of 50 chicks. All the birds of group I, III, V and VII were infected with 20,000 sporulated oocyst of *Eimeria tenella*. Birds of group I, III and V were treated with amprolium, at dose rate of 0.025 per cent in drinking water for two weeks,

Table - 6: Seasonal Incidence of coccidiosis in Poultry birds in and around Desari, Vaishali.

Season	Total no. of birds examined	No. of infected birds	Percentage of infected birds	$\chi^2_3$ d.f.
Spring (Feb-March)	112	12	10.71	
Summer (April-June)	142	18	12.67	
Rainy (July-Sept)	130	44	33.84	38.91**
Winter (Oct-Jan)	124	10	8.06	
Total	508	84	16.53	

<sup>\* \*</sup> Significant at P<0.01

Table - 7: Incidence of different species of coccidia of chickens in and around Desari, Vaishali (on the basis of postmortem examination):

Species of Eimeria	No. of infected birds	Percentage of infected birds	No. of non- infected birds	Total no. of birds examination	x <sup>2</sup> 4. d.f.
Eimeria tenella	43	8.46			
Eimeria necatrix	15	2.95			
Eimeria acervulina	12	2.36	424	508	68.49**
Eimeria maxima	8	1.56			
Other Eimeria spp.	6	1.18			
Total	84	16.53	424	508	

<sup>\* \*</sup> Significant at P<0.01

Fig. 3: Histogram showing seasonal incidence of coccidiosis in poultry birds in and around Hajipur.

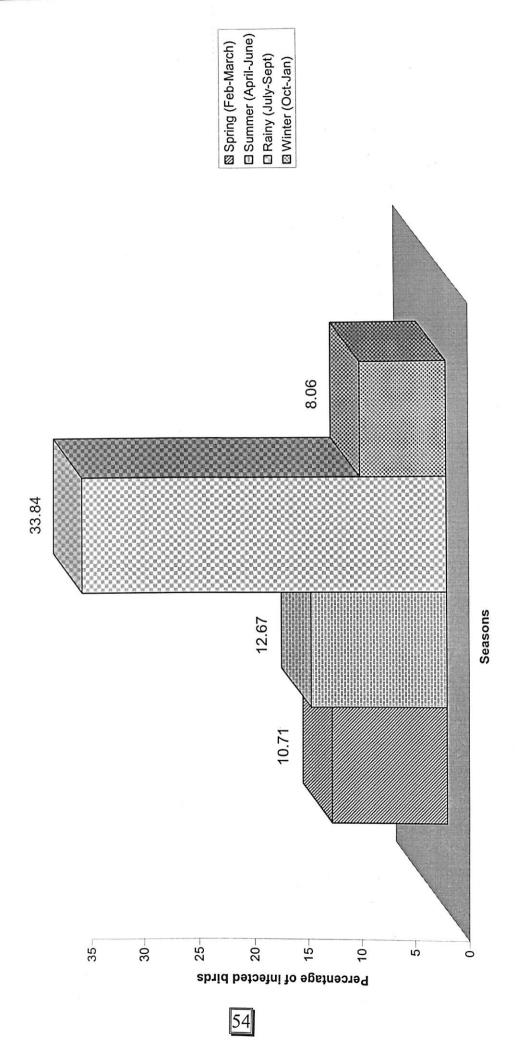


Fig. 4: Bar diagram showing incidence of different species of coccidia in chicken in and around Hajipur

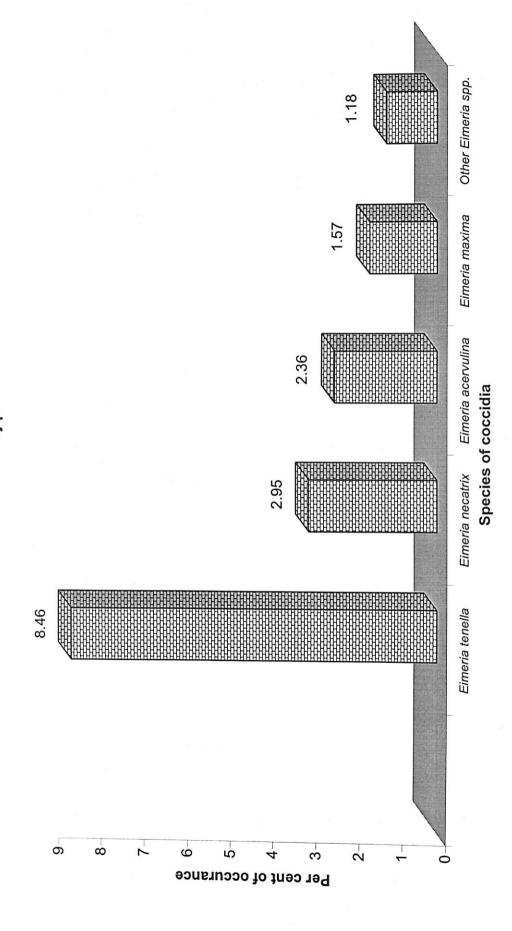


Table No.-8: Effect of anticoccidial agents on average body weight (gm) of birds infected with Eimeria tenella.

	No.	Treatment	1st week	2 <sup>nd</sup> week	3rd week	4th week	
	-	Infected Amprolium	49.32±0.11	84.20+0 10	110 25+0 00		om Week
	-	treated	(50)	(50)	110.25±0.08 (35)	139.89±0.08 25)	160.04±0.12 (25)
	II	Uninfected Amprolium treated	49.78±0.10 (50)	84.61±0.10 (50)	120.36±0.07 (40)	160.36±0.09 (30)	184.54±0.12 (15)
	III	Infected Sulphachloropyrazine treated	49.65±0.08 (50)	84.69±0.10 (50)	116.13±0.10 (37)	141.51±0.09 (27)	169.73±0.10 (27)
LS	IV	Uninfected Sulphachloropyrazine treated	49.69±0.08 (50)	85.06±0.08 (50)	120.07±0.11 (40)	161.15±0.11 (30)	185.26±0.12 (17)
	<	Infected Toltrazuril treated	50.27±0.09 (50)	85.21±0.10 (50)	118.41±0.08 (39)	150.70±0.10 (29)	180.43±0.16 (29)
	VI	Uninfected Toltrazuril treated	49.940.09 (50)	85.15±0.07 (50)	120.14±0.08 (40)	161.36±0.09 (30)	185.82±0.10 (16)
	VII	Infected untreated	49.79±0.11 (50)	85.09±0.07 (50)	99.94±0.11 (24)	130.14±0.29 (14)	154.38±0.17 (14)

<sup>\*</sup> Values in parentheses indicate number of birds under observations.

<sup>1.</sup> Infective inoculum administered on day 14th.

<sup>2.</sup> Challenge inoculum on day 28th.

5th week 4th week Age of birds (in weeks) 3rd week 2nd week 1st week 200 . . O 180 160 Body weight (gm) 20 8 140 20 9 40 58

Fig. 5 : Line diagram showing effect of anticoccidial agents on average body weight (gm) of birds infected

with Eimeria tenella.

## Effect of anticoccidial agents on mortality rate of birds infected with Eimeria tenella:

The result of the post-infection mortality rates in chicks have been summarized in table-9. It was observed that there was two percent mortality in toltrazuril treated (Group-V), eight per cent mortality in sulphachloropyrazine treated (Group-III) and 12 per cent mortality in amprolium treated (Group-I) birds. It was further observed that at the given dose, the efficacy in respect to survival rate was highest with in comparison to sulphachloropyrazine toltrazuril amprolium. However, a total of 26 out of 50 birds received infection, died of severe clinical coccidiosis recording the mortality rate of 52 per cent in infected untreated control group (Group VII). On the other hand, no morality was observed in birds of all the three negative control groups (Group II, Group IV and Group VI) which were only medicated without any infective inoculum of Eimeria tenella oocysts.

The results of the post challenge status in birds of both treated and untreated group have been summarized in table10.

It was observed that all the 25 surviving birds of the previously infected and treated birds of Group I (Amprolium treated), 37 surviving birds of Group III (Sulphachloropyrazine treated) and 29 surviving birds of Group V (Toltrazuril treated) withstood the challenge infection recording a cent per cent survival rate. On the other hand, it was observed that, 50 per cent mortality in uninfected sulphachloropyrazine treated (Group IV), 46.66 per cent mortality in uinfected toltrazuril

Table No.-9: Effect of anticoccidial agents on the mortality of chicks infected with 20,000 sporulated oocyst of Eimeria tenella.

			09						
VII	IA	V	VI	III	II	н		No.	Group
Infected untreated control	Uninfected Toltrazuril treated	Infected Toltrazuril treated	Uninfected Sulphachloropyrazine treated	Infected Sulphachloropyrazine treated	Uninfected Amprolium treated	Infected Amprolium treated			Treatments
50	50	50	50	50	50	50	per group	birds	No. of
0	0	0	0	0	0	0	Day	$\mathfrak{Z}^{\mathrm{rd}}$	Po
1	0	1	0	0	0	0	day	4th	st infe
16	0	0	0	Ю	0	3	Day	5 <sup>th</sup>	ction n
6	0	0	0	1	0	2	Day	6њ	Post infection mortality status
3	0	0	0	1	0	1	Day	7њ	y statu
0	0	0	0	0	0	0	day	8th	S
26	0	1	0	4	0	6		Mortality	Total
52	0	2	0	8	0	12	(%)	mortality	Rate of
48.00	100.00	98.00	100.00	92.00	100.00	88.00	(%)	Survival	Rate of

Table No.-10: Effect of anticoccidial agent on the mortality of chicks challenged with 40,000 sporulated oocyst of Eimeria tenella.

			19						
VII	VI	<	IV	Ш	II	Ι		No.	Group
Infected untreated control	Uninfected Toltrazuril treated	Infected Toltrazuril treated	Uninfected Sulphacloropyrazine treated	Infected Sulphachloropyrazine treated	Uninfected Amprolium treated	Infected Amprolium treated			Treatments
14	30	29	30	27	30	25	per group	birds	No. of
0	0	0	0	0	0	0	day	3rd	Po
0	0	0	0	0	0	0	day	4 <sup>th</sup>	st Chal
0	9	0	10	0	12	0	day	5th	lenge r
0	2	0	1	0	2	0	day	6th	Post Challenge mortality status
0	3	0	2	0	1	0	day	7 <sup>th</sup>	ty statı
0	0	0	0	0	0	0	day	8th	SI
0	14	0	13	0	15	0		Mortality	Total
0.00	46.66	0.00	43.33	0.00	50.00	0.00	(%)	mortality	Rate of
100.00	53.34	100.00	56.27	100.00	50.00	100.00	(%)	Survival	Rate of

treated (Group VI) birds. It was also observed that all the 14 surviving birds of infected untreated control group withstood the challenge infection and no mortality observed in this group.

## Effect of anticoccidiosis agents on lesion score in chicken infected with *Eimeria tenella*:

Lesion scoring was done on the basis of lesions observed in the caeca during post-mortem and post sacrifice examination. It was scored in the units of 0 to 4. A unit of 4 was taken as 100 per cent for lesion score, while a lesser degree of lesions were given scores of 3, 2, 1 and 0 as described under materials and methods.

In infected amprolium treated group (Group I) the average lesion score varied from 1.00 on day 7<sup>th</sup> post challenge to 1.07 on the 7<sup>th</sup> day post infection. However, the score was 1.00 in birds slaughtered on day 14<sup>th</sup> of post infection (Table-11).

In birds of group III, infected and sulphachloropyrazine treated group the average lesion score varied from 0.00 on day 7<sup>th</sup> post challenge to 0.50 on the 7<sup>th</sup> day post infection. However, the score was 0.22 in birds slaughtered on day 14<sup>th</sup> of post infection (Table-12).

In infected toltrazuril treated group (Group-V) the average lesion score was 0.12 on 7<sup>th</sup> day of post infection. There was no lesion observed on 14<sup>th</sup> day post infection as well as on 7<sup>th</sup> day post challenge (Table-13).

In infected untreated group (Group VII), mortality of birds due to coccidiosis was observed on day 4th, 5th, 6th and 7th post infection. The average lesion score in these birds recorded was

Table - 11: Effect of Amprolium on lesion score of birds infected with Eimeria tenella.

Batch of Bird sacrificed	Day of mortality/ sacrifice	No. of birds sacrificed	Mean lesion score	Protection against + lesion (%)
I	7 <sup>th</sup> Day of post infection	9+1*	1.07	73.25
II	14 <sup>th</sup> day of Post infection (Sacrifice)	10	1	75
III	7 <sup>th</sup> day post challenge (Sacrifice)	25 (All surviving)	0	100

<sup>\*</sup> Natural death due to cacidiasis.

Table - 12: Effect of Sulphacholoropyrazine on lesion score of birds infected with *Eimeria tenella*.

Batch of birds sacrificed	Day of mortality/ sacrifice	No. of birds sacrificed	Mean lesion score	Protection against lesion(%)
I	7 <sup>th</sup> day of post infection	9+1*	0.50	87.50
II	14 <sup>th</sup> day of post infection	10	0.22	94.50
III	7 <sup>th</sup> day of post challegnge	27	0	100

<sup>\*</sup> Natural death due to coccidosis.

Table - 13: Effect of Toltrazuril on lesion score of birds infected with Eimeria tenella.

Batch of birds sacrificed	No. of birds sacrificed	Day of mortality/ sacrifice	Mean lesion score	Protection against lesion (%)
I	7 <sup>th</sup> day Post infection	10	0.12	97.00
II	14 <sup>th</sup> day Post infection	10	0	100.00
III	7 <sup>th</sup> day Post challenge	29	0	100.00

Table - 14: Lesion score in birds of uninfected treated (Negative control) group (II,IV and VI) after challenge infection with Eimeria tenella sporulated oocyst.

Batch of birds sacrificed	Day of mortality/ sacrifice	No. of birds sacrificed	Mean lesion score	Protection against lesion (%)
I	7 <sup>th</sup> day Post infection	Group-II 10 Anprolium treated Group-IV 10 Sulphacholorpyrazine treated Group-VI 10 (toltrazuril treated)	No lesion	100
II	14 <sup>th</sup> day Post infection	Group-II 10 Group-III 10 Group-VI 10	No lesion	100
III	7 <sup>th</sup> day Post challenge	Group-II 15+1 (Amprolium treated  Group-IV 17+2 (Sulphacholoropyrazine treated	3	25 25
		Group-VI 16+3 (Toltrazuril treated)	2	50

Table - 15: Lesion score in Eimeria tenella infected untreated birds of positive control group (Group-VII).

Batch of birds sacrificed	Day of mortality/ sacrifice	No. of birds sacrifice	Mean lesion score	Protection against lesion(%)
I	5 <sup>th</sup> day post infection (mortality)	16	4.00	0.00
II	6 <sup>th</sup> day post infection (mortality)	6	4.00	0.00
. III	7 <sup>th</sup> day post infection (mortality)	3	4.00	0.00
IV	14 <sup>th</sup> day post infection (Sacrifice)	10	2	50.00
V	7 <sup>th</sup> day post challenge (Sacrifice)	14	1	75.00

4.00 and it varied from 2.00 on day 14 post infection to 1.00 on 7<sup>th</sup> day post challenge. (Table-15). Grossly there was severe involvement of ceaca showing, extensive haemorrhage and 3 to 4 times increase in its size.

No lesion was recorded in birds of group II, group IV and group VI which were slaughtered on 7<sup>th</sup> day and 14<sup>th</sup> day post infection. Whereas birds sacrificed on 7<sup>th</sup> day post challenge showed lesion scoring 2 to 3 (Table-14).

## Effect of anticocidials on O.P.G. of birds infected with Eimeria tenella.

Data regarding the oocyst shed by the birds in post-infection and post challenge is presented in table-16 and 17 respectively. Data shows that birds of all three groups (group I, group-III and V) which were infected with 20,000 viable sporulated oocyst of *Eimeria tenella* on day 14th started shedding oocyst in their faeces from fifth day of post-infection. Oocyst per gram of faeces (O.P.G.) estimated to be 16,000 on fifth day which reduced to 4,700 on eighth day in group I, 12,600 on fifth day which reduced to 4,800 on eighth day in Group III and 2,000 on fifth day which reduced zero on sixth day in group V. While in the control groups which were unmedicated O.P.G. was 48,000 and 98,000 on fifth and eighth day respectively.

Birds of all three negative control groups (Group II, IV and VI) which were not infected remain negative for any coccidian oocyst.

In the post challenge infection, data from table-17 reveals that the oocysts appeared in the faeces of birds on fifth to sixth

day post-challenge. In birds of group-I the O.P.G. was 4,000 on sixth day post challenge which fell down gradually to 1600 on eighth day.

Birds of all negative control group which did not have any prior exposure to *Eimeria tenella* infection started shedding oocyst on fifth day post-challenge e.g. a day earlier to infected group. O.P.G in theses group ranged 46,000 in amprolium treated group to 50,500 in toltrazuril treated groups of birds. O.P.G. value in these groups gradually rose and on seventh day reached to maximum of 69,000 in toltrazuril treated birds (Group VI).

Birds of group I (amprolium treated), group III sulphachloropyrazine treated and group V (toltrazuril treated) which had prior exposure to *Eimeria tenella* infection started shedding oocyst on sixth day post challenge e.g. a day latter. The maximum and minimum value of O.P.G. in these group was 4200 and 38,00 in group V and Group III respectively.

Birds of infected untreated group (Group VII) which reacted sharply to the infective inoculum of 20,000 oocyst of *Eimeria tenella* and suffered severely and recovered from clinical coccidiosis gave poor response to challenge infection. Birds of these group started sheeding oocyst on sixth day post-challenge, and O.P.G. gradually declined from the 800 on 6th day 400 in eighth day post-challenge.

## Effect of anticoccidial agents on average faecal score of birds infected with Eimeria tenella.

The results of average group faecal score from third to eight day post-infection and from third to eighth day post

Table No.-16: Oocyst per gram of faeces (O.P.G) of birds infected with 20,000 sporulated oocysts of Eimeria tenella.

				69			
VII	5	<b>V</b>		H	ш	-	Group No.
Infected untreated (Positive Control)	Uninfected Toltrazuril treated (Negative Control-III)	Intected Toltrazuril treated	Uninfected Sulphachloropyrazine treated (Negative Control –II)	Infected Sulphachloropyrazine treated	Uninfected - Amprolium treated (Negative Control –I)	Infected - Amprolium treated	Treatments
50	50	50	50	50	50	50	No. of birds
0	0	0	0	0	0	0	Zero day
0	0	0	0	0	0	0	O.P.G. level 2nd 4th
0	0	0	0	0	0	0	_
48,000	0	2,000	0	12,600	0	16,000	ring pos
86,000	0	0	0	6,200	0	6,900	t infectio
1,00,000	0	0	0	4,800	0	4,700	during post infection period  5th 6th 7th
98,000	0	0	0	3,200	0	3,800	8th
83,000	0	2,000	0	6,700	0	7,850	Mean Number O.P.G. observed

Table No.-17: Oocyst per gram of faeces (O.P.G) of birds challenged with 40,000 sporulated oocyst of Eimeria tenella.

				OL			
VII	VI	<	N	Ħ	П	-	Group No.
Infected untreated (Positive Control)	Uninfected Toltrezuril treated (Negative Control-III)	Infected Toltrezuril treated	Uninfected Sulphacloropyrazine treated (Negative Control –II)	Infected Sulphachloropyrazine treated	Uninfected Amprolium treated (Negative Control –I)	Intected Amprolium treated	Treatments
14	30	29	30	27	30	25	No. of birds
0	0	0	0	0	0	0	3rd
0	0	0	0	0	0	0	P.G. leve
0	50,500	0	47,000	0	46,000	0	O.P.G. level during post infection period  4th 5th 6th 7th
800	59,500	4,200	53,000	3,800	58,000	4,000	ost infect
500	69,000	3,100	63,000	1,700	65,000	2,900	tion perio 7 <sup>th</sup>
400	56,000	1,800	58,000	1,200	60,000	1,600	8th
566	58500	3033	55350	2233	57250	2833	Mean Number O.P.G.

challenge have been summarized in table 18 and 19, respectively.

The result of faecal score following the initial infection showed that lowest average score (0.33) was recorded in toltrazuril treated birds among the infected group, the sulphachloropyrazine treated birds showed slightly higher faecal score of 0.50, while the highest mean faecal score (0.66) was recorded in amprolium treated birds. No faecal score was obtained in birds of uninfected treated groups (Group II, IV and VI) while very high score (2.33) was observed in birds of infected untreated control group (group VII).

A reverse trend was observed while the surviving birds were challenged with 40,000 sporulated oocyst. During the post challenge infection period highest score of 1.66 was observed in birds of both sulphachloropyrazine and toltrazuril treated group of birds followed by 1.50 in amprolim treated birds. Birds of uninfected treated group (Group II, IV and VI) the score were 1.83, 2.00 and 1.83, respectively. However, no faecal score was observed in 14 surving birds of infected untreated control group (Group VII).



Table No.-18: Effect of anticoccidial agents on the faecal score of birds infected with 20,000 sporulated oocyst of Eimeria tenella.

	Group	Treatments	No. of		Faecal sco	re during post-challenge period	ost-challe	enge perio	₹.	Moon
			birds	3rd	4th	5th	6th	7th	8th	faecal
	ы	Infected Amprolium	<u>7</u> 0	0		)				score
	-	treated	50	0	<u> </u>	2	Н	0	0	0.66
		Uninfected								
	П	Amprolium treated	50	0	0	0	0	0	0	0.000
		Infected								
	III	Sulphachloropyrazine treated	50	0	ш	1	1	0	0	0.50
		Uninfected								
77	W	Sulphachloropyrazine treated (Negative	50	0	0	0	0	0	0	0.00
		Control-II)								
		Infected to Toltrazuril	л Э	>	•		)			
	_	treated	00	C		-	0	0	0	0.33
		Uninfected Toltrazuril								
	VI	treated (Negative	50	0	0	0	0	0	0	0.00
		Control-III)			1	-				
		Infected untreated				-				
	VII	control (Positive	50	0	2	4	4	ω	1	2.33
		control)								

Table No.-19: Faecal score of surviving birds of different treatment groups challenged with 40,000 oocyst of Eimeria tenella.

			EL				
VII	VI	V	IV	III	II	I	Group No.
control (Positive control)	Uninfected Toltrazuril treated (Negative Control-III)	Infected Toltrazuril treated	Uninfected Sulphachloropyrazine treated (Negative Control-II)	Uninfected Sulphachloropyrazine treated	Uninfected Amprolium treated (Negative control-I)	Infected Amprolium treated	Treatments
14	30	29	30	27	30	25	No. of birds
0	0	0	0	0	0	0	3rd
0	1	0	1	0	Ъ	0	Faecal score
	4	2	2	1	3	0	
0	ω	ယ	ယ	2	3	1	during post challenge period  5th 6th 7th
0	2	1	2	2	2	8	nge perio
0	1	1	1	1	1	2	d 8th
0.00	1.83	1.66	2.00	1.66	1.83	1.50	Mean faecal score





CHAPTER - V

# DISCIPLON





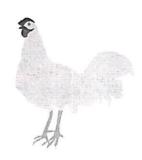


#### CHAPTER - V

# DESCENSION OF THE PROPERTY OF







CHAPTER - V

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### DISCUSSION

Geographical location of Bihar lies between 25.6N and 85.12 E. There are four seasons of the year. From beginning of July to end of September constitute rainy season, having average rain fall of 1.25 mm. April to August are the hot months of the year and December and January are the cold months. During summer, the environmental temperature goes up to 47°C, whereas winter temperature used to be as low as 4ºC. Atmospheric humidity level remains between 70 per cent in winter to 80 per cent in summer Hot and humid climate of the region is highly congenial for the growth and multiplication of coccidia, that is why the disease is more prevalent in Bihar. Data in table no. 4 and 5 showed the incidence of coccidiosis in blocks of district Vaishali, reveals that out of 508 bird screened, 84 (16.53%) were positive for coccidiosis on the basis of faecal sample examination. Earlier record on incidence of coccidiosis in Bihar by Mandal (1959) was only 8 per cent. Gupta (1971) from Mathura and Kalra et al, (1994) from Punjab, Haryana and Himachal Pradesh recorded the incidence of coccidiosis as low as 12.5 per cent and 3.29 per cent, respectively. However, Shashi Kala (2005) from Bihar recorded the incidence of coccidiosis as 16.54 per cent. Though the infection rate in these studies were low, there was report of as high as 35 per cent infection (Pohl, 1969) in Newzealand.

In the present study, the higher incidence rate of (27.96%) was observed in younger age group of two to 10 weeks old birds than adult (9.24%). In Canada, Lee and Onderka (1978) observed that 80 per cent incidence rate in broiler birds of three to seven weeks old.

Sinha (1965) reported that younger birds between the age of three weeks to three months were more susceptible than the birds over three month of age. It was also observed that the younger birds carry acute and severe type of infection while the older birds carry a chronic type of disease. Gupta, (1971) reported that death rate in zero to four weeks of age group was higher (56.7%) than adults within the age group of 12-16 weeks in which it was only 4.4 per cent. The Chi-square test also confirms the highly significant effect of different age group of birds on incidence of poultry coccidiosis. (Calculated x2 value 1 d.f = 19.09). Additionally highly significant (P<0.01) effect of different performing types of birds (layers and broilers) on the incidence of coccidiosis was observed (table-5) in which broiler chicks showed higher incidence (21.85%) of coccidiosis than the layers (10.50%). The findings were in accordance with observation of Deo (1988) who also found that the incidence of the disease was higher in broiler than the layers, though the younger birds were more susceptible to infection than the adult birds. Several authors have also reported that the outbreaks of coccidiosis were more and greater in number during spring season (Sinha, 1965, Gupta, 1971) though Lee

and Onderka (1978) from Canada and Oikava (1979) from Japan reported highest incidence of coccidiosis during rainy season and lowest in winter month of December. In the present study the seasonal effect on the incidence of coccidiosis presented in table-7 shows that the disease was highest during rainy season (33.84%) and lowest during winter (8.06%). The highly significant effect of season on the incidence of coccidiosis in poultry was established by Chisquare test. The calculated value of x2 for 3 d.f. was 38.91. It is presumed that higher ambient temperature coupled with humidity level higher facilitate sporulation the and development of oocyst in litter leading to increase in the incidence of disease during rainy season in India.

The data presented in the table further indicate that though four species of coccidia viz. Eimeria tenella, Eimeria necatrix, , Eimeria acervulina and Eimeria maxima are mainly involved in the causation of clinical coccidiosis in poultry in the region the predominant species affecting birds was Eimeria tenella (8.46%) followed by Eimeria necatrix (2.45%), Eimeria acervulina (2.31%) Eimeria maxima (1.57%) and other species of Eimeria constitute 1.18 per cent, respectively and the difference between the species was highly significant (P<0.01).

#### Chemotherapeutic efficacy of anticoccidials:

Out of the three anticoccidial agents viz: amprolium, sulphachloropyrazine and toltrazuril tested for chemothrerapeutic efficacy, toltrazuril when given at dose rate

of 7 mg/kg body weight in water was found to be most effective so far its effect on checking mortality rate is concerned. It provided 98 per cent protection against coccidiosis. Amprolium at the dose rate of 0.024 per cent in drinking water, showed 88 per cent protection to experimental infection with 20,000 viable sporulated occyst of Eimeria tenella. Sulphachloropyrazine at the dose rate of 0.03 per cent in drinking water, showed 92 per cent protection. It is worth noting that the Mogollon and Molina (1977) observed no effect of Amprolium on Eimeria tenella at a dose rate of 0.0125 per cent as the infected birds continued to shed oocysts in their faeces and lossed weight even after prolonged therapy. The present observation clearly indicate that the efficacy of the drug is highly dose dependent and as the dose was doubled the efficacy of the drug was increased.

The effect of challenge infection with 40,000 sporulated viable oocyst of Eimeria tenella in all the seven groups of brids (Table-12) further suggested that the birds which exposed to the infection earlier became immune and those which did not had prior exposure to infection suffer clinically and showed 50 per cent mortality in uninfected amprolium treated, 43.3 per cent in sulphachloroparazine treated and 46.66 per cent in uninfected toltrazuril treated group of birds. Whereas, no mortality was recorded in infected treated group [group I, III, VI) as well as in the surviving birds of infected untreated group (Group VII). This may be due to the fact that these birds which

suffer clinical coccidiosis during initial infection developed immunity and could withstood the subsequent challenge infection. These observation was in close agreement with the finding reported by Varga (1988), Varga and Laczay (1988), Munoz et al. (1988) and Jin et al. (2003).

The efficacy of the three anticoccidials were also assessed on the basis of body weight gain. The data presented in table-8 revealed that the maximum increase in weight was attained in birds of uninfected toltrazuril treated and minimum growth was observed in birds of infected and amprolium treated group. Whereas weight gain was markedly affected in birds of infected untreated group (VII). In a similar study, Lakkundi et al. (2002b), and Pathak and Kapoor (1998) observed higher weight gain in birds treated with toltrazuril whereas Merlanti and Quaglio (1986) observed that body weight gain in toltrazuril treated birds was comparable to weight gain in birds treated with sulphachloropyrazine.

Lesion score is considered as the visible effect of the drug on coccidiosis. Data regarding the effect of different anticoccidials on lesion score in birds infected with Eimeria tenella is presented in table-13 to 17. The results of the present investigation were in agreement with the findings of Yvore et al. (1980), Badsture et al. (1986) and Huang et al. (2002) who observed lesser lesion score in birds having prior exposure to coccidiosis. In infected amprolium treated group (Group I) the lowest lesion score was 1.00 on 14th day post

post infection. No lesion was observed on 7th day of post challenge. In birds of infected sulphachloropyrazine treated group (Group III) the lesion score was only 0.50 on 7th day post infection whereas comparatively lesser lesion score 0.22 was observed 14 days after infection. No lesion score was observed in these birds during post challenge period. Birds of infected and toltrazuril treated group (group-VI) which showed lower mean lesion score (0.12) on day seventh of post infection but no lesion was observed on 14th day post infection as well as on seventh day of post challenge. Thus it was observed that birds which had got pre exposure to infection developed solid immunity and did not suffer again during post challenge infection.

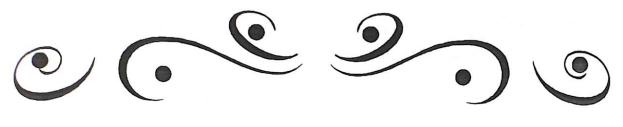
Faecal score is not a correct indicater of level of protection by a particular anticoccidial agent. This is because in many cases an increase in faecal score has been observed at the initial stage even in successfully treated birds. In the present investigation faecal score commenced from fourth day of post infection in all infected treated groups as well as in infected untreated group VII. After challenge infection the faecal score was observed from fourth day post challenge in all the uninfected treated group whereas no faecal score was observed in groups of birds which had prior exposure of infection (i.e group I, V and VII).

Oocyst count, play an important role in assessing the efficacy of anticoccidials. Result of present investigation showed that oocyst started appearing in faeces of the birds from fifth day post infection onwards. Birds of amprolium and sulphachloropyrazine treated group in which oocyst appeared on fifth day post infection showed 16,000 and 12,600 oocyst per gram (O.P.G) of faeces-Birds of toltrazuril treated group also shed oocyst on fifth day of infection but the O.P.G count was only 2000 then gradually dropped to zero.

During challenge infection (Table-19) the birds of group I, III and V which had prior exposure to infection started shedding oocyst a day later i.e. sixth day post challenge in contrast to fifth day in group II, IV and VI which received infection first time during challenge. These group also showed higher O.P.G. count in comparison to birds of previous group.

On the basis of overall results it could be suggested that toltrazuril was highly effective in controlling the caecal coccidiosis in poultry followed by sulphachloropyrazine and amprolium, and is also cost effective as it gives better result in checking mortality and development of lesion at very low dose. It was observed that in order to maintain the efficacy of a compound as a potent anticoccidial the dose of the compound needs to be marginally increased after a frequent internal. This gives sufficient indication of the development of drug resistance in the parasite.

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CHAPTER - VI

# SUMMARY AND CONCLUSION



#### SUMMARY AND CONCLUSION

In the present study a total of 508 poultry birds from the local poultry farms in blocks of Vaishali district and the birds slaughtered in the vicinity of Hajipur were screened for presence of coccidian parasite.

It was observed that 22.96 per cent of young birds and 9.24 per cent adult birds found to shed coccidial oocyst in their faeces. Infection was more common (21.85%) in broiler birds than the layer birds (10.50%). It was found that *Eimeria tenella* was the most prevalent (8.96%) species followed by *Eimeria necatix* (2.15%), *Eimeria acervulina* (2.36%), *Eimeria maxima* (1.57%) and all other species constitute only 1.18 per cent. The infection was more common in rainy season (33.84%) followed by summer (12.67%), Spring (10.71%) and winter (8.06%). Season has a significant role in the incidence of the coccidiosis.

of anticoccidials use Continuous to coccidiosis cause development of drug resistance in the parasite which required higher dose of anticoccidials for control of the disease making it economically unviable to poultry farmers. Therefore, it became imperative to screen drugs used vizamprolium, commonly some aginst sulphachaloropyrazine and toltrazuril the drug resistente strain of Eimeria tenella is of prime importance in controlling the coccidiosis. The literature regarding the efficacy

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The trial was conducted on day old chicks reared under coccidia free condition. The chicks were fed starter ration and were infected with 20,000 sporulated viable oocyst of Eimeria tenella on 14th day and treated with amprolium, sulphachloropyrazine and toltrazuril on 4th day post infection and challenged with 40,000 sporulated viable oocyst of the same batch at the age of 28th day. Based on the mortablity rate, weight gain, lesion score, O.P.G. and faecal score during post infection as well as post-challenge period, efficacy of drug, was assessed.

The result showed that there was great variation in anticoccidiocidal effect of drugs viz: amprolium at a dose rate of 0.024 per cent in drinking water, sulphachaloropyrazine at a dose rate of 0.03 per cent in drinking water, and toltrazuril at a dose rate of 7 mg per kg body weight in drinking water. The mortality rate in amprolium treated group was recorded as 12 per cent, but in sulphachloropyrazine treated group it was eight per cent, whereas in toltrazuril treated group it was two per cent. The birds of infected treated groups (Group-I, III and V) which were exposed to infection became immune and withstood challenge infection of 40,000 sporulated oocyst of Eimeria tenella and survival rate to challenge infection was 100 per cent in all the three infected treated groups. The birds of infected untreated control group which suffered severe

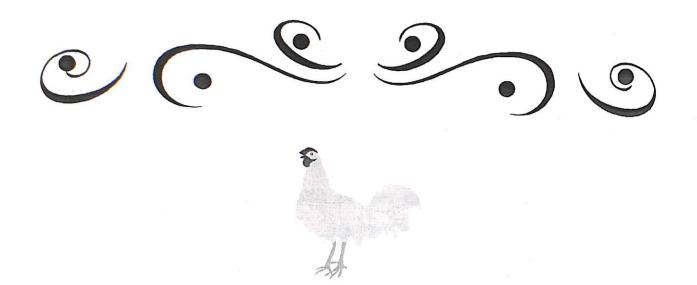
clinical coccidiosis and survived, developed immunity to the challenge infection more readily than the birds of all three infected treated groups which suffered less. These immune birds even checked the development of subclinical disease and lesion. In birds of this group the tissue reaction was minimum leading to insufficient development of immunity. The performance of toltrazuril was better in comparision to amprolium and sulphachloropyrazine in checking lesion formation by Eimeria tenella. Hence oocyst count was also much lower in toltrazuril treated birds over amprolium and sulphachalorpyrazine treated birds. A reverse trend was observed during challenge when the surviving birds were challenged with 40,000 sporulated oocyst of Eimeria tenella the drug which performed poorly to the initial infection showed better performance in terms of lesion score and O.P.G. count. This could also be attributed to the development of immunity in the birds which reacted sharply to the initial infection and suffer clinically.

With respect to body weight gain birds treated with toltrazuril showed slight increase in their body weight after the commencement of treatment while the groups of birds treated with sulphachaloropyrazine and/or amprolium did not show any conspicuous weight gain. These indications were sufficient to conclude that non of these drugs have any positive role in the body weight gain.

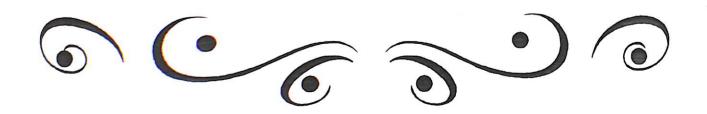
On the basis of these observations it could be concluded that toltrazuril was highly effective in controlling the caecal coccidiosis in poultry followed by sulphachaloropyrazine and amprolium, and is also cost effective as it gives better results in checking mortality as well as development of lesion at a very low dose of 7 mg/kg body weight.

Amprolium and sulphchaloropyrazine though effectively checked the infection were slower in action and required longer period for the regain of health, whereas toltrazuril was quick in action in effectively controlling the infection before the cause of tissue damage. Hence taking into consideration of economic viability and the performance it is suggested that toltrazuril which gave better performance in short time should be preferred in the treatment of ceacal coccidiosis in broiler chicks.

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