CHAPTER # 5

D I S C U S S I O N
Rabbits, besides their use as laboratory animals, are raised for a variety of commercial purposes viz., wool, meat and fur. They are efficient converters of vegetable protein into high quality animal protein. In India, rabbit farming for wool and meat has developed into an important industry and it has brought handsome returns to the rabbit rearers (Tripathi et al. 1995) these returns are often affected by the outbreak of various diseases, especially coccidiosis, in this animal. Coccidiosis caused by *Eimeria* species not only results in tremendous economic losses to rabbit industry (Leysen et al. 1989) but is also responsible for major losses in poultry industry world wide Shirley (1992); Lillehoj and Trout (1993). It is thus an emerging disease of increasing importance in commercial rabbitries. (Licois et al., 1990)
A study on prevalence of *Eimeria* infection in rabbits of Kashmir valley was conducted during Mar. 2004-Feb. 2006. A total of 9576 faecal samples were collected from the host population comprising of different breeds. The overall incidence revealed that 66.26% of the rabbit population harboured *Eimeria* infection. This is in agreement with the study of Bhat and Jithendran (1996) who reported a prevalence of 64% of *Eimeria* from the rabbit population of Himachal Pradesh. Perusal of literature reveals that prevalence of rabbit coccidiosis was quite variable from as low as 8.5% (Jiang, 1981) to 100 percent (Wang & Tsai, 1991). The present study revealed that coccidia was a very common protozoan inhabiting gastrointestinal tract of rabbits and maximum incidence was observed in rabbits reared under intensive system of management. This coincides with the study of Meitei (1988) who also reported higher incidence of coccidiosis in flocks reared under intensive system of management in Bihar.

**Season-wise prevalence:**

In the present study, intensity of infection was maximum in Spring (97.04%) followed by Summer (78.75%), Autumn (51.89%) and Winter (45.47%) respectively, which is attributed to climatic conditions favouring sporulation of oocysts causing frequent infection by contaminated feed and drinks during Spring and Summer seasons. Lower incidence (40.84%) during Winter months was also noticed by Meitei (1988) as compared to 66.35% during rainy months, which co-relates with the present findings.
wise details reported by Gurpratab and Khahra (1997), also revealed maximum incidence in monsoon (July to October) as 39.26% and summer 34.07% (March to June) and minimum in winter season (23.31%). Low incidence of coccidia during Winter season was also reported by Gill and Ray (1960); Holland et al. (1988) and Pilar et al. (2005) which also are in agreement with the present study. Thus, seasonal prevalence was lowest during winter (45.47%) as humidity and tendency to huddle together in the cold season is attributed to the incidence of coccidiosis during these months.

**District wise prevalence:**

The prevalence of coccidiosis was more in district Baramullah (79.41%) followed by district Srinagar (66.35%). The high percentage of infection in these units is attributed to more damp conditions and poor sanitation. General hygienic measures indicated that rabbits given a dry rather than moist pellets, washed fresh vegetables and plenty of fresh water as was apparent from the rabbits of district Anantnag, especially in Rambirpur govt. rabbit breeding farm, in these conditions coccidiosis is unlikely to appear. This is in agreement with Raida et al., (2001) who reported high incidence of disease in those breeding and rearing establishments where sanitation was poor. The level of infection based on OPG was related to managemental practices (Jithandran & Bhat, 1996). Similar findings on high prevalence of coccidial infection in unhygienic conditions have also been reported by Coudert (1989) and placid et al. (1993) which also favours the present results.
Age-wise prevalence:

In the present study the age wise prevalence of coccidia was found to be more in weaners (86.97%) and growers (65.61%) than adults (39.43%) particularly in the animals reared under intensive farming conditions. This was due to the overcrowding stress and repeated infection by ingestion of feeds and fodders contaminated by the carriers. The prevalence of *Eimeria* in adults was found to be less as they have acquired resistance by continuous and low grade of natural infection. This present study is in accordance with Wang and Tsai, (1991) who reported that the infection rate was highest in young rabbits (80.7%) than in adults (28.2%). Krishna and Vaid (1987), reported that young ones suffer severely from acute infection. Further, infection rate decreased as the age of rabbit increased (Hagen, 1958). *Eimeria* infection is more pathogenic in young rabbits and has led to increased mortality (Gomez-Bautista *et al.*, 1987). Jithendran and Bhat (1996), reported that the overall infection/mortality due to coccidiosis was higher in weaners (46%) than in the growers (24%) and adults (6%). Sena and Suryanarayana (1996), recorded highest incidence of infection in rabbits below 3 months of age. The age wise prevalence of *Eimeria* in rabbits also agrees with the observations of ogedengbe *et al.*, (1994) and Ajuwape *et al.*, (1999). These authors reported that young animals are more susceptible to coccidia infection than older animals.
Sex-wise prevalence:

Out of the total adults (5447) screened for presence of *Eimeria* parasite in the present study, there were 1709 (54.56%) does and 1046 (45.18%) bucks infected showing thereby preponderance of disease in doves than bucks. This study revealed that females mostly became carriers and act as the source of infection for the younger stocks. Similar observations have been made by Sanyal and Srivastava (1986), who reported that the prevalence of coccidia was 54.05% infection in females and 48.93% in males, respectively. Wang and Tsai (1991), also reported that the infection rate in female rabbits was high (28.2%) compared to males (19.5%). High percentage prevalence in females as compared to males was also reported by Sha-meem and Devada, 2005. However, Sena and Suryanarayana (1997), showed insignificant variation (p>0.05) on the influence of sex on coccidiosis. This disparity was due to the confinement of these breeds individually in cages with regular cleaning and use of coccidiostats.

Mixed Infection:

During the course of present study it was observed that 5699 (89.80%) were harbouring mixed infection with three or more than three species of *Eimeria*. Such multispecies infection is in agreement with Ajayi *et al.* (1987); Kasim and Alshawa (1987) and Catchpole and Norton (1989). In an unusual case Andres (1969), reported nine species of *Eimeria* from a single infected rabbit. According to Pellerdy (1965), one individual rodent can be a host to 5 or more *Eimeria* species, which favours the findings of
our observations. Mixed infection was also reported by Gurpratap and kahra (1997); Sanyal and Srivastava (1986); Jithendran and Bhat (1996); Jain (1988); and Meitei et al., (1988) in different rabbit breeds.

Speciation:

During the present study the criteria used for identifying different species of *Eimeria* were prepatent period, sporulation time, morphological characters, host specificity and inhibition of different sites of host by the parasite (Tyzzer, 1924). The observations revealed the *Eimeria magna*, *E. media*, *E. exigua*, *E. piriformis*, *E. perforans*, *E. irresidua*, *E. intestinalis* and *E. stiedai* were involved in infecting the rabbits of different age groups. Same criteria were adapted by Pellerdy (1974); Gill & Ray (1960); Sanyal and Srivastava (1986); Jain (1988); Meitei (1988) and Coudert (1989). The species identification was made by exogenous study based on sporulation time, morphology and morphometry of sporulated oocysts purified after centrifugal flotation by Catchpole and Norton, (1979) & Maff (1984). Morphological features of both unsporulated oocysts, sporulated oocysts and sporulation time were taken into consideration for speciation of *Eimeria* species in rabbits by Akram, 1991; Jithendran, 1995 and Hobbs, 1998.

**Therapeutic control with Sulphaquinoxaline:**

To control the clinical coccidiosis in T1 group, Sulphaquinoxaline @ 2 gms. / litre of water was given. The observations recorded included.
**Pre-patent period:**

The observation on pre-patent period after ingestion of one lakh sporulated oocysts showed that the oocysts of all species of *Eimeria* appeared in the faeces of both infected and treated rabbits. The prepatent periods for different species of *Eimeria* remained more or less same in both T<sub>1</sub> and C<sub>1</sub> groups. The present findings on pre-patent period of all seven species of *Eimeria* were in the range and conformity of the reports given by Kheysin, 1947; Pellerdy, 1974; Sanyal and Srivastava, 1986 and Meitei, 1988.

**Clinical symptoms:**

The characteristic signs and symptoms of coccidiosis as observed in rabbits of groups T<sub>1</sub> and C<sub>1</sub> with 1 lakh sporulated oocysts induced acute coccidiosis in most of the animals. The severity of the disease was obvious in two of the rabbits of group C<sub>1</sub> which died of the infection on 27<sup>th</sup> and 28<sup>th</sup> day PI.

This showed that the infective challenge of the present experiment was sufficient to cause clinical coccidiosis. This is in agreement with the study of Meitei, 1988 who also reported that rabbits were anaemic, dull, depressed, off fed & a few died of coccidia after rabbits were challenged with one lakh of oocysts. Similar findings on clinical symptoms were reported by Vanparijs et al., (1989), when rabbits were infected with 100,000 sporulated oocysts of intestinal species. However, Junior et al.(1996) reported similar symptoms of coccidiosis by challenging the
rabbits with 50,000 sporulated oocysts. Tassi & Puccini (1984), in another study used 2, 00,000 oocysts of mixed species of *Eimeria* & recorded death of most of rabbits with similar symptoms. This proved that the dose of infection was high to cause death of rabbits.

**Discharge of Oocysts:**

The present study on discharge of oocysts indicated that the mean OPG in C1 group was significantly higher than T1 group which indicated that the drug has affected the multiplication power of the *Eimeria* resulting into reduced number of oocysts in faecal counts. Thus; the drug can be successfully advocated for the control of coccidial infection in rabbits. This present study is in full agreement with placid *et al* (1992) who reported a sharp decline in OPG count which reached to minimum levels by day 40 of the experiment. Laha *et al.*, (1999) also observed drastic reduction in OPG by using Sulphaquinoxaline. Tassi and Puccini (1984), in another study reported significant reduction in faecal counts by using sulphaquinoxaline @ 125ppm which favours the present findings.

**Body weight:**

The observations on body weight gain indicated that there was not much difference of weight gain in animals of T1 and C3 groups but both groups differed significantly from C2 group. This indicated that the severity of infection in animals of group C2 did not allow them to grow normally. From this observation, it was concluded that the drug used is highly
DISCUSSION

Efficacious in reducing the effects of coccidiosis in rabbits. Similar findings on weight gain were observed by Pakandl, 1986; Meitei, 1988 and Niedzwiedek et al, 1990, thus supported the present findings. Ajuwape et al., (1999) & Tassi & Puccini (1984) reported similar findings on body weight in rabbits.

PROPHYLACTIC CONTROL WITH DICLAZURIL AND MADURAMYCIN:

Clinical symptoms:

The characteristic symptoms of coccidiosis as observed in rabbits of groups T2, T3 and C2 were mucoid diarrhoea, depressed, off-fed, visible paleness of mucous membrane, inactiveness and thirst. These symptoms are similar to the reports of workers observed during naturally induced coccidiosis in sheep and goat by Pande, Bhatia and Chauhan, 1972. Diarrhoea, debility, anorexia, weakness and arched back symptoms in naturally infected rabbits have been reported by Meitei et al., (1988) and Sudhan, 1991 which support the present findings.

Discharge of oocysts:

The mean OPG in T2, T3 and C2 group animals before treatment were in the nearest range indicating thereby, the severity of infection before treatment was similar in all the groups. But after the drug administration, there was abrupt fall in the OPG of T2 & T3 group rabbits which proved that Diclazuril @ 1 ppm and Maduramycin @ 2ppm can be successfully
used to control coccidiosis in rabbits. The present study is in conformity with the results reported by Kintzel and Hasslinger (1995) and Junior, et al., (1996). Vanparijs et al., (1989) reported that oocyst output was reduced to zero and faecal aspect was normal when Diclazuril was given @ 1 & 2 ppm which also supports the present findings. Polozowski, (1993) reported similar findings by using Maduramycin @ 2 ppm as anticoccidial drug, thus favouring the present findings. Therefore, the drugs used were 100% effective in reducing oocyst output & faecal scores.

**Body Weight:**

From the observations on body weight it was concluded the Diclazuril @ 1ppm and Maduramycin @ 2ppm were highly efficacious in reducing effects of coccidiosis and increasing production in growing rabbits. It is also desirable that during the growing stage of rabbits, a feed mixture having either a coccidiostat or coccidiocidal drug be made available to the animals before the infection affects the production. Although compensatory growth was observed in infected untreated (IUT) rabbits of C2 group, the economic advantage of efficient body weight gain was more pronounced in T2 & T3 groups when the infection was prevented with Diclazuril and Maduramycin medication. Thus prevention of coccidiosis in rabbits treated with Diclazuril and Maduramycin may be an answer to increase production with less investment. This study is in agreement with Vanparijs et al., (1989) & Polozowski (1993), who reported significant increase in body weight gain of IT (infected treated) compared to severe weight gain depression in IUT (infected untreated) rabbits. The drugs used were Diclazuril and
Maduramycin, respectively. Similar were the findings of Kintzel and Hasslinger, 1995 and Balicka et al., 1992 on body weight gain.

**Haematological Studies:**

The haematological changes were recorded in respect of Haemoglobin (g/dl), Packed Cell Volume (PCV %) and Differential Leucocyte count (DLC %).

**Haemoglobin:**

In this study haemoglobin (g/dl) compared in different groups revealed higher value in $C_3$ group (UIUT) during the same period of observation. The reduced value of haemoglobin in IT group and IUT groups can be attributed to tissue damage and consequent blood loss caused by endogenous stages of parasite. Our findings are in agreement with those reported by Novinskaya et al., (1983), Meitei (1988), Sena and Suryanarayana (1997) and Tambur et al. (2001) who reported an increase in Hb values of treated groups. However in another study Lineburg (1987) reported an insignificant decrease in haemoglobin in rabbits infected with *E. stiedai*. This disparity in the results could be due to the single species involved and the size of inoculum.
**Packed cell volume:**

The PCV values of both IT and UIUT groups remained same which reflected that the drugs used viz., Sulphaquinoxaline, Diclazuril and Maduramycin were helpful in keeping the animals in healthy conditions. Our observations are in total agreement with Meitei (1988), Sena and Suryanarayana (1997) and Tambur et al. (2001) who also observed an increase in PCV values in rabbits. This study indicated that during coccidiosis tissue damage and consequent blood loss caused by endogenous stages of parasite cause decrease in PCV%.

**DIFFERENTIAL LEUCOCYTE COUNT:**

**Neutrophils:**

In this study neutrophilia was observed to have positive correlation with the severity of the disease. The increase in circulating neutrophilia is probably due to a well established defense mechanisms (phagocytosis) involving degranulation of leucocytes. This study is in agreement with Tambur et al. (2004) who also reported neutrophilia in infected rabbits. Besides, Svanbaev (1969), Rama et al. (1978) Shommein et al. (1980) and Basharat (1988) in lambs observed neutrophilia infected with different *Eimeria* species.
Lymphocytes:

The observed decrease in lymphocytes is due to the increase in neutrophils. This is also so because we have calculated the number of differential count of neutrophils as percentage and not as absolute number of cells per cubic mm of rabbit blood. Therefore, the fact remains that the observed decrease in lymphocytes is due to increase in percent neutrophils. This study indicated that the acute nature of disease involves specifically neutrophils as there has been no significant difference of other types of white cells in all the groups. However, Tambur et al (2004) reported increase in monocytes and basophils while no change was found in eosinophil values of infected rabbits.

Total protein and albumin

Total protein and albumin values in IT and UIUT groups were significantly higher than IUT groups. The decrease of total serum proteins and albumin levels might be due to exfoliation of mucosal epithelium of intestine and bile duct which resulted in loss of proteins and albumin along with leakage of plasma. Thus the concentration of total protein is altered by changes in plasma volume with alteration of albumin / globulin ratio as is the case in protein losing enteropathy. It may also be a consequence of disordered absorption of the products of protein digestion during the course of disease. It is assumed that the degree of protein loss, further digestion and absorption may be incomplete with increase in the faecal nitrogen loss. In such cases, the loss of protein from the circulation leads to
hypoproteinaemia and hypoalbuminaemia. A similar trend was also reported by Novinskaya et al., (1983), Coudert et al. (1978) and Jiunn & Cicero (1995) thus co-relating with the present findings. Sena & Suryanarayana (1997) also reported decreased total serum proteins & albumin levels in rabbits infected with coccidia. Tambur et al., (1998) also reported significant decrease (P< 0.05) in total protein & albumin, when rabbits were infected with mixed species of *Eimeria*.

**Electrophoretic findings:**

While studying the electrophoretic pattern of the proteins in both IT & IUT in comparison with UIUT group it was found that there was an increase in levels of $\alpha$ (alpha), $\beta$(beta), $\beta_2$ and $\gamma$ globulins in infected rabbits as compared to healthy groups. It is because antitrypsin forms about half the $\alpha_1$ globulin and is responsible for most of the observable change in the intensity of the electrophoretic band. It is the major inhibitor in the plasma of proteolytic enzymes including trypsin, catalase, plasmin and thrombin and an increased pattern in $\alpha_1$ globulin reflects the inflammatory status, thus it belongs to a group of plasma protein collectively known as acute phase reactive proteins. Therefore, because of disease process loss of proteins into the intestines and because of the haemorrhagic shock resulting in the protein losing enteropathy and destruction of the hepatocytes, so synthesis of proteins is low. It can be explained that hyperglobulin is due to acute phase phenomenon and as a consequence of parasitic infection. On electrophoresis although they mainly have mid $\gamma$ mobility, small amounts are also found in the extreme slow $\gamma$ region and on the other hand stretching across the $\beta$ to
the $\alpha_2$ zone. This finding positively correlates with the results discussed earlier by Coudert et al. (1978) who observed principle changes in total protein -20% alpha-globulin +7%, betaglobulin +5.2%, gamma globulin +3.6% and albumin - 24%. Similar were finding of Jiunn and Cicero (1995), who reported hypoproteinaemia and hypoalbuminaemia in rabbits with *Eimeria* species.

**Histopathology:**

The rabbits of IUT group developed severe congestion of serosal layer with haemorrhagic spot in the intestine and liver. This indicated that 1 lakh dose of infective challenge was sufficient to cause clinical coccidiosis in younger rabbits leading to death of two rabbits. The gross and histopathological findings of enlarged liver with whitish nodules, hyperplasia of the bile duct epithelia with infiltration of mononuclear cells and presence of oocysts in the bile duct epithelia were as previously described by Martine and Yvone (1974), Ogendengbe et al., (1994), Jibike et al. (1995) and Ajuwape et al., (1999). The thickness of basement membrane might have a defensive role in warding off the coccidial infection. this study is in agreement with Darzi et al., 2006. Likewise, the histopathological observations of intestine revealed serosal congestion, pin point haemorrhage at several places and presence of large number of gametocystic stages of protozoa within crypts of liberkuhn, as reported by Krishna and Vaid (1987) and Meitei (1988). The clinical signs of weight loss resulting from anorexia and liver damage are similar to those reported by Peeters et al. (1982), Gomez-Bautista(1987) and Ajuwape et al. (1999).