

EIMERIA MAGNA : THE EFFECT OF VARYING INOCULUM SIZE ON THE COURSE OF INFECTION IN ANGORA RABBITS.

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ABSTRACT : The effect of graded infection of *Eimeria magna* oocysts was studied in German Angora rabbits. A dose level of 2×10^4 sporulated oocysts was found to be satisfactory for getting a good oocyst yield and maintaining

the general health of the host. The changes in the body weight, diarrhoea and mortality in the host indicated that this coccidial species was moderately pathogenic to Angora rabbits.

RESUME : *Eimeria magna* : effets de la taille de l'inoculum employé pour une infection expérimentale chez le lapin angora.

Les auteurs ont testé l'efficacité d'un gamme de doses d'*Eimeria magna* pour provoquer une infection coccidienne contrôlée chez des lapins angora allemand (de 1 à 10×10^4 oocyste sporulés/animal). La dose de 2×10^4 oocystes

sporulés a été considérée comme la plus satisfaisante en ce sens qu'elle permet une "bonne" excréation fécale d'oocystes sans altération de la santé des lapins inoculés. Les variations de poids, la fréquence des cas de diarrhée et de mortalité, indiquent qu'*Eimeria magna* a une pathogénicité modérée chez le lapin angora.

INTRODUCTION

Coccidiosis caused by different species of the protozoan parasite, *Eimeria* is one of the important diseases of rabbits and is a major cause of morbidity and mortality (LEBAS *et al.*, 1986). It mainly affects the intestine. Only one species causes hepatic lesions. A survey of rabbit farms in the Kangra valley of Himachal Pradesh revealed that *Eimeria magna* alone or in association with *E. media* and *E. perforans* was the main cause of intestinal coccidiosis in Angora rabbits reared in this region (BHAT and JITHENDRAN 1994). Of these three species, *E. magna* shows the highest pathogenicity to rabbits (LEBAS *et al.*, 1986). The aim of the present study was to determine in Angora rabbits the effect of graded infection doses of *E. magna* sporulated oocysts on various clinicoparasitological parameters, including oocyst multiplication rate, and to quantify a dose of infection suitable for conducting experimental studies on this host-parasite system.

MATERIALS AND METHODS

The faecal samples were collected from naturally infected animals in various rabbitries and were processed for preparation of infective coccidial cultures. A suspension of these faecal samples was prepared in 2% potassium dichromate solution for the preservation and sporulation of oocysts. The oocysts were sporulated by incubating in this solution at 27°C

in a B.O.D. incubator for 120 hours. A few sporulated oocysts with typical morphology of *E. magna* were picked out and propagated in a coccidia-free German Angora rabbit (6-8 weeks old). The faeces of this rabbit was collected during patency and processed for harvesting and sporulation of *E. magna* oocysts. These sporulated oocysts were further multiplied in a few more rabbits of the type described earlier for the ultimate preparation of a pure infective culture for experimental studies. Standard techniques were used for the isolation, concentration and purification of *E. magna* oocysts harvested from experimental animals (PRICE and REED, 1970). The faecal suspension (1 g per 30 ml water) was strained through a sieve and the filtrate was centrifuged for 2 min. at 1,500 rpm. The supernatant fluid was discarded, the sediment mixed with clean water and the suspension centrifuged for 2 min at 1,500 rpm. This procedure was repeated until the supernatant fluid was clear. After this the sediment was mixed with saturated sodium chloride solution and centrifuged at 1,500 rpm for 2 min. The top layer was skimmed off from various tubes, pooled and mixed with 2% potassium dichromate solution for preservation and sporulation. After sporulation the oocysts were enumerated, diluted and stored at 4°C in petri dishes. This culture of *E. magna* sporulated oocysts, prior to its administration, was washed free of potassium dichromate with distilled water and used for quantitative infection studies. The enumeration of oocysts was carried out by the modified MCMASTER technique (MAFF, 1977).

Angora rabbits (German strain) used for the study were procured from Angora Rabbit Breeding Farm of

Table 1 : Experimental design and oocyst output.

Groups	I	II	III	IV	V	VI
No of animals	4	4	4	4	4	4
Dose of sporulated oocysts given (x 10 ⁴)	1.0	2.0	5.0	7.5	10.0	0
Total mean (± SD) oocyst output (x 10 ⁶)	5.25 ± 0.33	10.5 ± 0.58	12.0 ± 0.35	12.5 ± 0.65	11.5 ± 0.89	0
Mortality	nil	nil	nil	nil	one	nil

Himachal Pradesh State Animal Husbandry Department. Twenty-four coccidia-free rabbits of either sex, aged 8–10 weeks, were randomly distributed into 6 groups of 4 animals each. The mean weight and age of the animals was, as far as possible, kept identical. The animals were housed individually in all-wire cages (60 x 40 x 40 cm) equipped with a plastic screen underneath for faecal collection. Cages were supported by angle-iron supportters about 80 cm above the concrete floor. They were placed in a concrete building with good ventilation having an ambient temperature of 18–25°C, and were shielded from all external contamination. Feed concentrate and greens (tree leaves) viz. mulberry (*Morus alba*) and biul (*Grewia optiva*) were fed *ad libitum* to the animals in feeders, periodically cleaned and sterilised by autoclaving. Tree leaves, besides being a good source of protein and crude fibre for Angora rabbits (SINGH *et al.*, 1984 ; SINGH and NEGI, 1986) were preferred to other greens to avoid contamination. Water was offered *ad libitum* in waterers which were also cleaned and sterilised like feeders. The animals in 5 different groups (I to V) were inoculated *per os* with varying single infection of *E. magna* sporulated oocysts (Table 1). Groups VI rabbits were kept as uninfected-control. They were studied for 6 weeks ; oocysts per gram of faeces (OPG) counts were recorded daily and body weight weekly during the period of study.

Statistical analysis of data was done using ANOVA (one way) test. A confidence level of 95 % or more was considered to be significant.

RESULTS AND DISCUSSION

The prepatent period varied from 7–10 days and patency was for a short period of 5 days in all the infected groups. The characteristics symptoms of the infection during the patency period were loose faeces, slight loss of weight and shedding of oocysts. A mild diarrhoea and moderate mortality was also observed in Group V rabbits. The change in the body weight of the experimental groups (I–V) was non significant till second week post-infection, when compared to control animals (Group VI). The differences between Group V

and VI–V and other experimental groups became significant from third week post-infection. The differences between each remaining experimental group and control group became significant only by the end of the experiment. The daily OPG counts varied from 1.05–4.5 x 10⁴ in the infected groups during patency. The total mean oocyst output values in the infected groups which was highest in Group IV rabbits (Table 1), were significantly different except for Group III vs IV and III vs V. The infected animals gradually recovered during post-patency phase and their growth returned to near normal by the end of the experiment.

In European countries, *E. intestinalis* is considered to be one of the most pathogenic coccidial species in the rabbit (LEBAS *et al.*, 1986). In India, *E. magna*, *E. media* and *E. perforans* are the main cause of intestinal coccidiosis in rabbits (SANYAL and SRIVASTAVA, 1986 ; KRISHNA and VAID, 1987 ; JAIN, 1988 ; MEITEI *et al.*, 1989 ; CHANDRA and GHOSH, 1990 ; BHAT and JITHENDRAN, 1994). The results of the present investigations show that there is no concomitant increase in the oocyst output at higher doses of infection (5 x 10⁴ and above). This indicates that the power of multiplication of oocysts is not proportional to the number of oocysts administered to the animals and it decreases after reaching a particular level. The ability of oocysts to multiply in the host varies with the coccidial species and the type of animals infected (LICOIS *et al.*, 1992). Earlier workers have reported a yield of 10⁸ oocysts in New Zealand White rabbits infected with 10⁴ oocysts (COUDERT, 1989 ; DROUET-VIARD *et al.*, 1994). In the present study, the low total oocyst output in all the infected rabbits may be due to the existence of a partial immunity in these animals at the time of inoculation. This immunity could have been acquired by them as a result of low level exposure to the parasite immediately during post-weaning period in the rabbitry. The effect of the breed and strain of the host on lowering the oocyst yield also cannot be ruled out in the present case.

Thus, we can infer that for multiplying this species and carrying out experimental studies on *E. magna* infection, the dose level of 2 x 10⁴ sporulated oocysts is sufficient for getting a relatively good yield of oocysts in this breed of rabbit without causing any

Table 2 : Changes in body weight (kg) in infected and control groups of Angora rabbits (mean ± SD).

Group	Week post-infection						
	0	1	2	3	4	5	6
I	1.23 ± 0.13	1.25 ± 0.12	1.26 ± 0.11	1.28 ± 0.11	1.30 ± 0.12	1.36 ± 0.13	1.44 ± 0.13
II	1.21 ± 0.04	1.25 ± 0.04	1.29 ± 0.04	1.31 ± 0.03	1.36 ± 0.04	1.39 ± 0.03	1.45 ± 0.04
III	1.22 ± 0.10	1.26 ± 0.10	1.30 ± 0.10	1.33 ± 0.11	1.38 ± 0.11	1.43 ± 0.13	1.47 ± 0.14
IV	1.21 ± 0.10	1.23 ± 0.09	1.25 ± 0.09	1.28 ± 0.09	1.31 ± 0.10	1.35 ± 0.09	1.39 ± 0.10
V	1.22 ± 0.06	1.20 ± 0.06	1.14 ± 0.08	1.16 ± 0.07	1.17 ± 0.07	1.21 ± 0.05	1.26 ± 0.05
VI	1.21 ± 0.11	1.26 ± 0.12	1.32 ± 0.13	1.38 ± 0.12	1.46 ± 0.13	1.55 ± 0.14	1.65 ± 0.13

debility or mortality in them. It can also be concluded that taking the criteria of body weight change (Table 2), diarrhoea and mortality into consideration, this strain of *E. magna* as per the classification of COUDERT (1989), can be categorised as moderately pathogenic to German Angora rabbits.

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REFERENCES

- BHAT T.K., JITHENDRAN K.P., 1994. Preliminary studies on the prevalence and control of coccidiosis in Angora rabbits. In : *Cell Biology of Parasitic Protozoa* (in press).
- CHANDRA D., GHOSH S.S., 1990. Incidence of diarrhoeal infections in rabbits. *Indian J. Anim. Sci.*, **60**, 801-803 .
- COUDERT P., 1989. Coccidia and intestinal coccidiomorphs : Some peculiarities of rabbit coccidiosis. In : *Proc. Vth International Coccidiosis Conference, Tours (France), INRA Paris ed.*, 481-488.
- DROUET-VIARD F., COUDERT P., LICOIS D., BOIVIN M., 1994. Essai de vaccination contre la coccidiose a *Eimeria magna* à l'aide d'une lignée précoce. In : *Proc. VIèmes Journées de la Recherche Cunicole, La Rochelle, France, 6-7*, 38-43.
- JAIN P.C., 1988. Prevalence and comparative morphology of sporulated oocysts of eight species of *Eimeria* of domestic rabbits in Madhya Pradesh. *Ind. J. Anim. Sci.*, **58**, 688-691.
- KRISHNA L., VAID J., 1987. Intestinal coccidiosis in Angora rabbits - an outbreak. *Ind. Vet. J.*, **64**, 986-987 .
- LEBAS F., COUDERT P., ROUVIER R., de ROCHAMBEAU H., 1986. The rabbit : husbandry, health and production. *Animal Production and Health, vol. 20, F.A.O. ed., Rome*, 118-127 .
- LICOIS D., COUDERT P., DROUET-VIARD F., BOIVIN F., 1992. *Eimeria perforans*, and *E. coecicola* : multiplication rate and effect of the acquired protection on the oocyst output. *J. Appl. Rabbit Res.*, **15**, 1433-1439 .
- MAFF, 1977. Manual of Veterinary parasitological techniques. *Technical bulletin, 18, HMSO*, 69-76.
- MEITEI H.M., PRASAD K.D., SAHAI B.N., ANSARI M.Z., 1988. Coccidial fauna of rabbits in Ranchi (Bihar). *J. Vet. Parasitol.*, **2**, 145-148.
- PRICE C.J., REED J.E., 1970. Practical Parasitology. General laboratory techniques and parasitic protozoa. *UNDP-FAO publ., Rome*, 44-81 .
- SANYAL P.K., SRIVASTAVA C.P., 1986. Subclinical coccidiosis in domestic rabbit in semi-arid part of Rajasthan. *Ind. J., Anim. Sci.*, **56**, 224-225 .
- SINGH B., GOEL G.C., NEGI S.S., 1984. Effect of supplementing mulberry (*Morus alba*) leaves *ad libitum* to concentrate diets of Angora rabbits on wool production. *J. Appl. Rabbit Res.*, **4**, 156.
- SINGH B., NEGI S.S., 1986. Effect on wool production of supplementing black locust (*Robinia pseudoacacia*) and biul (*Grewia optiva*) leaves *ad libitum* to a concentrate diets for Angora rabbits. *J. Appl. Rabbit Res.*, **9**, 159-163 .