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EGG OUTPUT OF GRAPHIDIUM STRIGOSUM (NEMATODA) IN LOW-LEVEL PRIME INFECTION OF RABBITS

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Abstract. Rabbits of Moroccan local breed were infected with larvae of *G. strigosum* obtained from a wild strain (100 larvae/animal) which had undergone two different durations of storage at +4 °C. A long storage (10 months) induced the inhibition of the larval development at a low-rate. A shorter storage of 3 months permitted the development into adults: the prepatent period was 5 weeks and the patent period lasted over 21 weeks post infection. The egg output expressed in eggs per gram was negatively correlated to the dry matter of faeces.

An appreciation of the daily egg output of nematodes is difficult. The variations from day to day are important and a clear estimation of the chronological evolution is not easy. These variations are partly due to sampling errors and partly due to technical errors as it was reviewed by Kloosterman (1971). Experimental infestations are usually realized with high doses of larvae. The utilized strains have undergone particular manipulations: storing of larvae at +4 °C for a certain time, development from eggs into infective larvae under laboratory conditions for several generations. Those two types of handlings interfere probably with the development of the worms; Ford (1971) has demonstrated that fresh and stored larvae induce differences in the immunological reactions and Martin et al. (1957) had shown the influence of the size of larval intake on the duration of pre-patent period for *Graphidium strigosum*. In the present paper, the evolution in time of the egg output of *G. strigosum*, is evaluated for a wild strain which underwent a storage and for a low dose of infective larvae. In order to reduce the variability of the observed egg output, several types of calculations have been tried and two concomitant factors are taken into account: weight of daily excreted faeces and their dry-matter. The influence on the egg output of 2 durations of larval storage is considered.

MATERIAL AND METHODS

Infective third-stage larvae. The larvae of *Graphidium strigosum* were obtained from adult females collected from the stomach of a naturally infected rabbit in the Rabat area.

Animals. They all originated from the same rabbit-farm from small rabbits aged 5.5 months which had not experienced previous infection with intestinal helminths. In each experiment the rabbits belong to the same litter.

Parasitological techniques. Eggs per gram of faeces (E.P.G.) were estimated by McMaster method using magnesium sulfate (density 1.20) as floatation solution. The daily estimates were made on 3 g samples of faeces. At necropsy the content of the stomach in its totality was examined. The larvae in the mucosa were counted after peptic digestion of the mucosa.

Experiment 1. Two rabbits were infected with 100 larvae each. These larvae had undergone 3 months storage at +4 °C. The E.P.G. and dry-matter were done every day for 21 weeks and the animals were then necropsied.

Experiment 2. Four rabbits were infected with 100 larvae each. The duration of larval storage was 10 months (at +4 °C). The daily E.P.G. were measured for 16 weeks and the animals were then necropsied.

RESULTS

Experiment 1

The prepatent period lasted five weeks. The results concerning the observed part of the patent period are presented in Table 1. At necropsy, the first animal harboured 91 adult parasites and the second — 40. The sex-ratio (female/male) was 1.05. No immature worms were recovered from the stomach lumen or mucosa.

Experiment 2

No egg was excreted during the period of observation. At necropsy only 4th-stage larvae in the mucosa were recovered, respectively 2, 2, 3, 6.

DISCUSSION

The prepatent period of 5 weeks seems shorter than it was reported by various authors. According to Flynn (1973), the duration of the prepatent period for *Graphidium strigosum* is 12 days, whereas Martin et al. (1957) give a value of 7 weeks. The prepatent period seems to be in fact more than one month. The differences observed between the results of Martin et al. (1957) and our results might be mostly due to the level of the infective doses: 100 in our work and 5 000 or 350.000 in these authors' paper. Nevertheless, the duration of this period is much longer than it usually is in trichostrongylids. *G. strigo-*

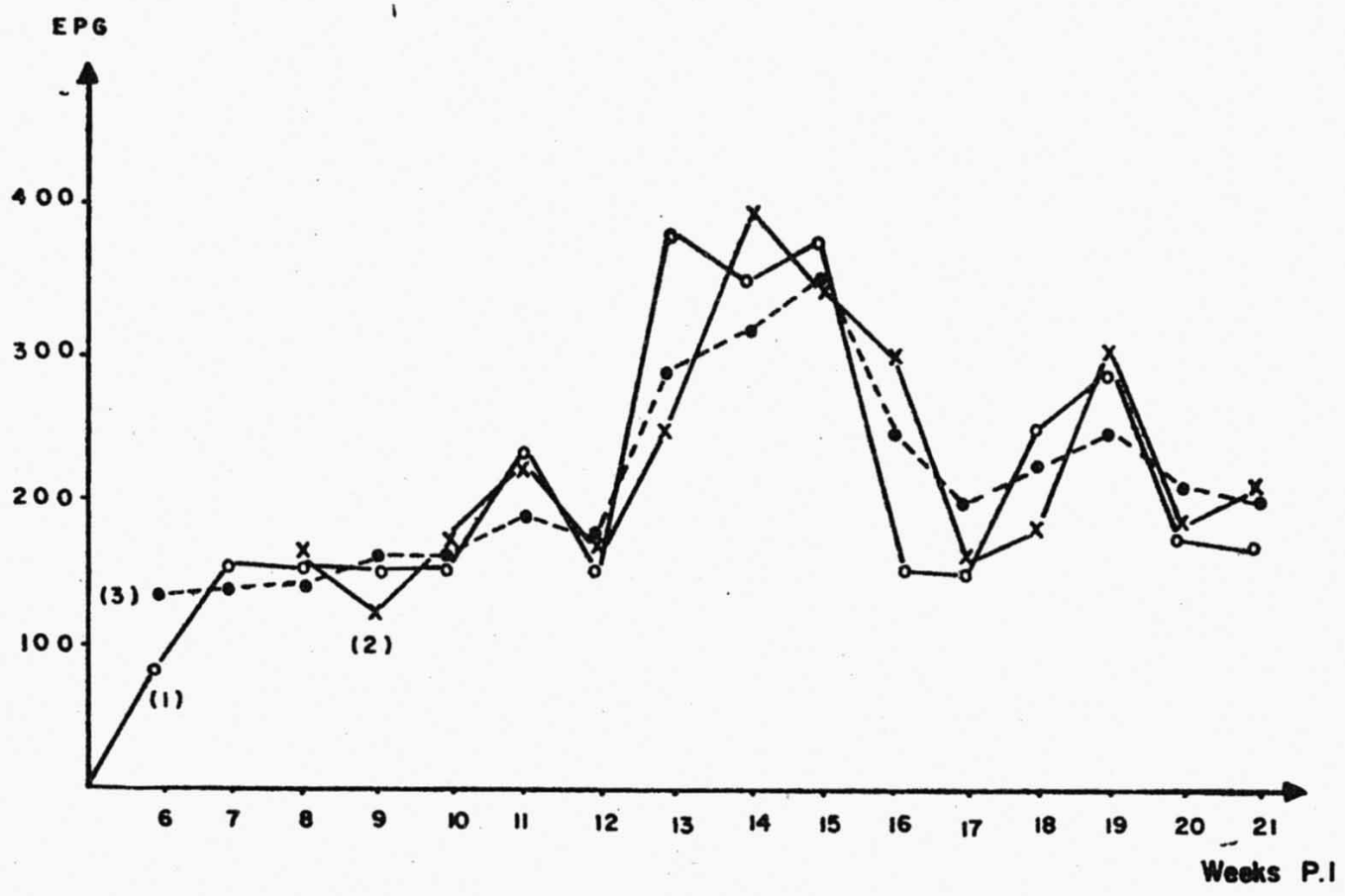

Fig. 1. Egg output of *Graphidium strigosum*. (1.) Weekly mean of daily E.P.G. (2.) Weekly mean of daily moving means of E.P.G. established on a 3-week cycle. (3.) Exponential smoothing ($\alpha = 0.5$) on weekly means.

Table 1. Evolution of E.P.G., weight of excreted faeces and their dry-matter

Average daily weight of faeces (in grams)	Dry matter (per cent)	Average daily E.P.G	Number of weeks post-infestation
—	—	80 (128)	6
210	59.3	156 (108)	7
185	49.6	154 (160)	8
135	55.7	154 (114)	9
125	51.1	158 (123)	10
141	49.8	237 (263)	11
135	42.6	158 (112)	12
116	38.5	391 (208)	13
79	38.2	340 (149)	14
159	46.7	373 (92)	15
104	45.5	151 (136)	16
125	65.5	150 (113)	17
127	50.9	249 (135)	18
108	61.4	282 (180)	19
130	58.2	173 (122)	20
160	60.6	182 (158)	21

Data between brackets are the standard deviation

sum which belongs to the same evolution line as *Ostertagia* spp. (Durette-Desset and Chabaud 1977), has then a different pattern in prolificity than the systematically related *O. circumcincta* whose preparent period is approximately 3 weeks. The prepatent period can be delayed when the infective larvae have undergone long storage at +4 °C, due to the phenomenon of inhibition of the larval development. According to Eysker (1974), neither storage nor repeated infections provoke inhibition in a large scale. We also obtained a low inhibition rate (2—6 % of the infective dose) but it could be of epidemiological importance as the fourth larval stage was the only surviving stage of *G. strigosum*.

The average weekly E.P.G. value includes much of chance variations as it can be seen from the standard deviation recorded in Table 1. The real evolution of the egg output is consequently unclear. The variability due to sampling of faeces and McMaster technique could account for about 50 % of the chance variations as it was tested; the increase in the number of faeces samples would than be of little value. Only statistical manipulations could reduce the variability of the observed data. The Taylor's (1965) method was inconvenient to our data as no obvious link could be demonstrated between the variance and mean of E.P.G. Two other methods, as described in Philips and Blomme (1973), moving averages and simple exponential smoothing were utilized. Moving averages in daily values established in a 3-week rythm and exponential smoothing (smoothing constant: 0.5) in weekly means are shown in Fig. 1. The last method seems to be more efficient in reducing the chance variability. The maximum output occurs between the 13th and 16th week post infection. The ability of egg hatching lasts long (at least 16 weeks) and the prolificity per day and per female is still important (420 eggs) at the end of the 21st week post infection.

As the lowest values for average daily weight of faeces (D.W.F.) and average daily dry-matter (D.D.M.) are encountered when the E.P.G. values are high, the influence of the volume of faeces and its consistency with E.P.G. was examined. A step-wise regression established in the 3 sets of weekly values showed that the E.P.G. were correlated to the D.D.M. = —5.53 DDM + 562 ($r = -0.53$). On the contrary, the daily E.P.G. is related positively to the daily weight of faeces (Spearman rank test: $P = 0.01$) at the period of maximal output. Two factors seem to influence the level of the E.P.G. in *G. strigosum:* the weight of faeces on daily egg production and the dry matter of these faeces on weekly egg production.

ЯЙЦЕКЛАДКА НЕМАТОДЫ *GRAPHIDIUM STRIGOSUM* (NEMATODA) В КРОЛИКАХ С НИЗКИМ ПЕРВИЧНЫМ ЗАРАЖЕНИЕМ

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Резюме. Кроликов, происходящих из местного марокканского племени, заражали личинками *G. strigosum*, полученными от дикого штамма (100 личинок/животное), хранившимися разное время при температуре 4 °С. Долговременное хранение (10 месяцев) вызывало торможение развития личинок. После трехмесячного хранения личинки развивались до зрелости. Препатентный период был 5 недель и патентный более 21 недели после заражения. Яйцекладка, выраженная в количестве яиц на грамму, находилась в отрицательной связи со сухим веществом осадка.

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EOSINOPHILIA IN EXPERIMENTAL TOXASCARIASIS (VISCERAL LARVA MIGRANS) IN WHITE MICE

One of the most common clinical symptoms of tissue helminthoses is the change in the blood picture, a high eosinophilia, which is one of the diagnostic features of these diseases. This paper deals with eosinophilia in experimental toxascariasis in white mice.

A total of 168 white mice were infected with eggs of *Toxascaris leonina* in the doses of 1500, 2500 and 3500 eggs. The method used was described in the paper by Prokopič and Klabanová (Čs. Epidem. 29: 171—176, 1980). Blood samples for serological studies and blood

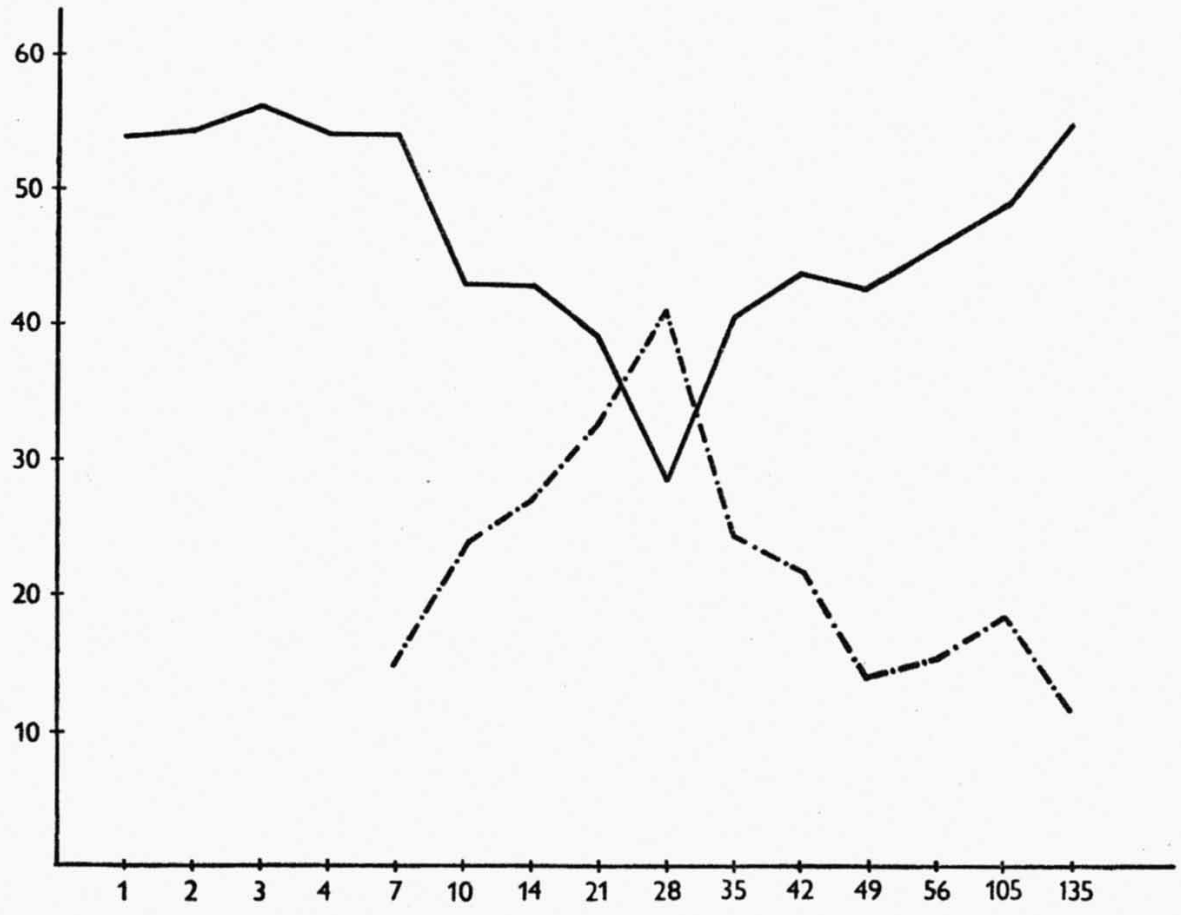

Fig. 1. Course of eosinophilia in experimental infection with *Toxascaris leonina*. Abscissa — number of days, ordinate — % of blood cells, ——— lymphoid cells, .—.—.— — eosinophilic cells.