

TRICHOSTRONGYLUS COLUBRIFORMIS: RELATIONSHIP BETWEEN AGEING OF INFECTIVE LARVAE, INFECTIVITY AND EGG PRODUCTION BY ADULT FEMALE WORMS

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Résumé

TRICHOSTRONGYLUS COLUBRIFORMIS: RELATIONS ENTRE VIEILLISSEMENT DES LARVES INFESTANTES, POUVOIR INFESTANT ET PONTE DES VERS ADULTES FEMELLES. — Pour étudier les relations entre la ponte et le pouvoir infestant chez *Trichostrongylus colubriformis*, des larves infestantes ont été conservées jusqu'à 22 semaines à 24 °C. Après différents temps, les larves ont été administrées à des moutons et le nombre de parasites ayant atteint le stade adulte a été déterminé cinq semaines après l'infestation. La ponte a été évaluée sur les mêmes lots d'animaux par coproscopie à partir de la 3^e semaine d'infestation. Le pouvoir infestant est maximum et constant pendant les six premières semaines de conservation puis diminue régulièrement à partir de la 9^e semaine. Inversement, le nombre d'œufs produits par chaque femelle est stable pendant les six premières semaines puis double à partir de la 9^e semaine et reste au même niveau jusqu'à 22 semaines de conservation.

Trichostrongyle infective larvae are capable of surviving for a long time in unfavourable conditions. Important physiological changes occur during ageing, which involve a decrease in infectivity, the rapidity and size of which depends upon the species of larvae and the storage conditions.

With *Heligmosomoides polygyrus*, a mouse parasite, it has been shown (Kerbœuf, 1978a; Mallet and Kerbœuf, 1984) that this decrease in infectivity varies according to time and temperature of storage of larvae and that, in the same species, females developing from young infective larvae excreted fewer eggs than those established from old infective larvae.

With *Trichostrongylus colubriformis* a similar decrease in infectivity has been observed (Ciordia *et al.*, 1966; Herlich, 1966; Herlich and Ryan, 1970) using guinea-pigs and rabbits as experimental hosts for the parasite. No study of infecti-

vity has been performed using sheep, the natural host for the parasite.

The aim of the present experiment was to ascertain for *T. colubriformis*, the consequences of ageing for infective larvae stored at 24 °C. Infectivity and egg production of adult female worms have been studied with experimentally infected lambs.

Materials and Methods

Trichostrongylus colubriformis infective larvae

Infective larvae were obtained from faeces collected from lambs reared indoors, free of parasites and infected orally with a single dose of 30000 infective larvae of a pure strain of *T. colubriformis*.

In order to obtain a sufficient number of larvae for the experiment, mass cultures were performed. Faeces were

incubated in plastic tanks (20 × 25 cm) in a controlled environment chamber at a temperature of 24 °C and with constant humidification.

After a ten-day incubation period, third-stage larvae were collected in tanks of the same dimensions using a modified Baermann apparatus (Gruner *et al.*, 1980).

Infective larvae were stored in the dark at 24 °C. To ensure sufficient oxygenation suitable for good preservation of larvae, they were stored under 1 cm of water in plastic cell-culture bottles with a surface of 175 cm² and at a concentration of no more than 3000 larvae per ml.

In order to supply larvae of different ages for experimental infection at the same time, four batches of two lambs were infected at intervals of three weeks and the larvae obtained from each batch were stored individually.

Experimental infections

Infectivity was assessed using Prealpes and Berrichon lambs weighing an average of 23 kg. For each batch of larvae, the animals were given a single oral dose of 20000 third-stage larvae. The experiment was performed with nine batches of five animals as homogeneous as possible in weight and sex.

Three series of infections were carried out on different dates. In the first series, three groups were infected with 0, 3 and 6 week-old larvae respectively; in the second series four groups of animals were given 9, 12, 14 and 22 week-old larvae respectively and finally two groups were infected with 19 and 20 week-old larvae (table 1).

Estimation of infectivity

In order to study both worm development and female egg production, the animals were slaughtered only five weeks after infection. The small intestine was removed and frozen (Hubert, 1980) for a later count of parasites.

Before examination, the intestines were thawed, then

opened along their whole length and washed. The contents of the intestine were adjusted to two liters with water and worms were counted in a 100 ml aliquot taken in four parts of 25 ml.

Estimation of female egg production

Faeces were taken from each animal twice a week at fixed times from day 15 after infection to slaughtering. Egg counts were performed using a modified McMaster technique (Raynaud, 1970).

Results

Infectivity of third-stage larvae

Results showed that infectivity varied according to the time of storage. This was maximum and constant (68 %) during the first 6 weeks and then decreased regularly to reach 12 % after 22 weeks of storage (fig. 1).

Fecundity of adult females

Egg production was estimated in two ways: (i) calculation of egg excretion by parasitized sheep expressed in eggs per gramme of faeces and (ii) calculation of egg production per female parasite.

The total egg excretion was maximum for 9 to 12 week old larvae at which stage the number of adult worms had just begun to decrease due to the decrease in larval infectivity (fig. 2).

When the number of eggs excreted was considered with respect to the number of females (fig. 3) a similarity appeared to exist between certain batches of the same series, which were therefore combined in order to increase the

Table 1. — Detail of experimental procedure.

Infective larvae (age in weeks)	Breed	Animals		Weight (kg) (mean ± SD)
		Sex		
		male	female	
0	Prealpes	2 ^a	3 ^a	22 ± 2
3	Prealpes	2	3	22 ± 2
6	Prealpes	2	3	22 ± 2
9	Berrichon	1	3	22 ± 3
	Prealpes	0	1	33
12	Berrichon	1	3	23 ± 4
	Prealpes	0	1	27
14	Berrichon	1	2	23 ± 4
	Prealpes	0	2	26 ± 2
19	Prealpes	5	0	25 ± 2
20	Prealpes	5	0	25 ± 1
22	Berrichon	1	3	25 ± 5
	Prealpes	0	1	29

a: number of animals

number of data for the series. The validity of this apparent similarity was verified.

From a variance analysis with complete blocks design it was shown that no significant differences existed between (i) batches 0, 3 and 6 weeks, (ii)

batches 9, 12 and 14 weeks, (iii) batches 19, 20 and 22 weeks. Therefore, the results were grouped as follows: "group A", 0 to 6 weeks; "group B", 9 to 14 weeks; "group C", 19 to 22 weeks.

Table 2. — Variance analysis with complete blocks design between batches 0 to 6 weeks; 9 to 14 weeks and 19 to 22 weeks.

	Sum of squares	Freedom degrees	Mean square	F	Signifiant
Between cases	4292623	14	306616	14.91	P < 0.001
Between batches	3987684	2	1993842	96.97	P < 0.001
Between days	146774	4	36693	1.78	N.S.
Interaction	158165	8	19771	0.96	N.S.
Residual	4318034	210	20562	—	—
Total	8610657	224	—	—	—

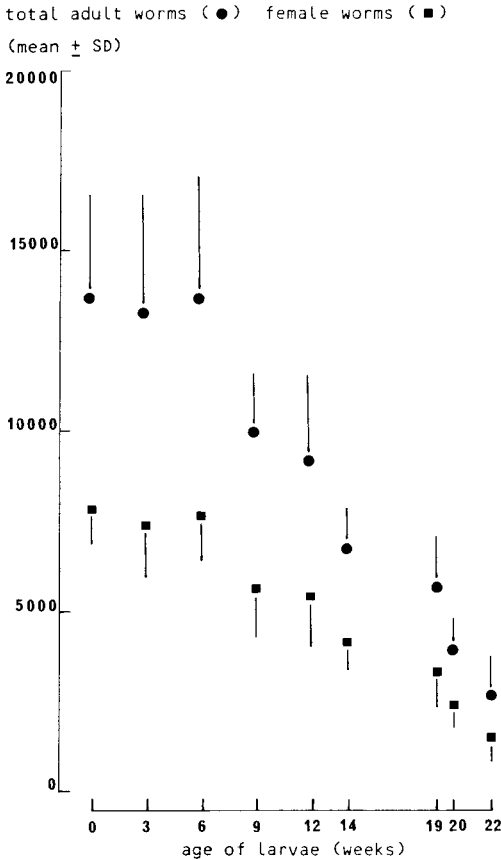


Fig. 1. — Decrease in infectivity of third stage larvae when time of storage increases; mean values of worm counts from 5 animals each given 20 000 infective larvae.

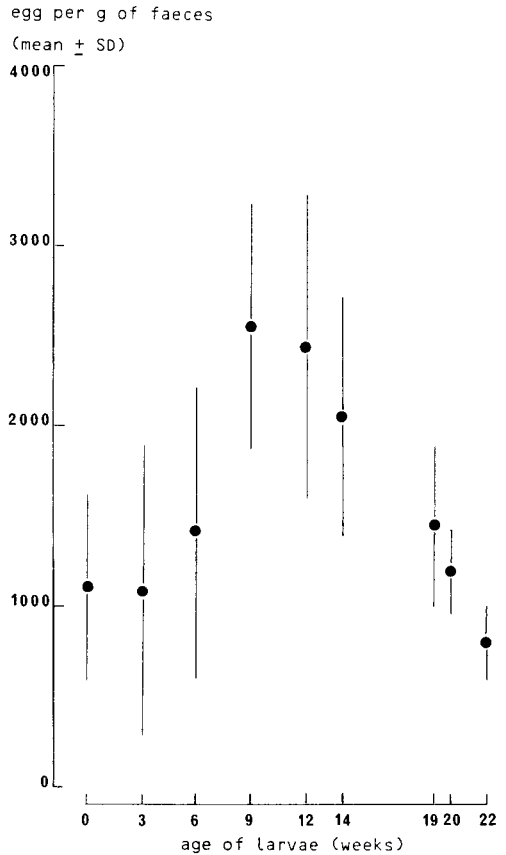


Fig. 2. — Total egg excretion of lambs given 20 000 infective larvae of different ages.

A further variance analysis with complete blocks design showed that highly significant differences exist between groups A, B and C (table 2).

To examine the fluctuations due to individual samples of faeces a comparison was made between the last five samples (between the 24th and 34th days post infection). No significant differences were noted proving that a plateau in egg production did exist during this period. The comparison of mean values by Scheffe's method showed a significant difference ($P < 0.0001$) between (i) groups A and B, (ii) groups A and C, but no significant difference between groups B and C.

Egg production remained constant with 200 eggs excreted per 1000 females and per gramme of faeces for larvae 0-6 weeks old and then doubled for larvae of 9 weeks and older (fig. 3).

Discussion

The differences in infectivity noted in this paper

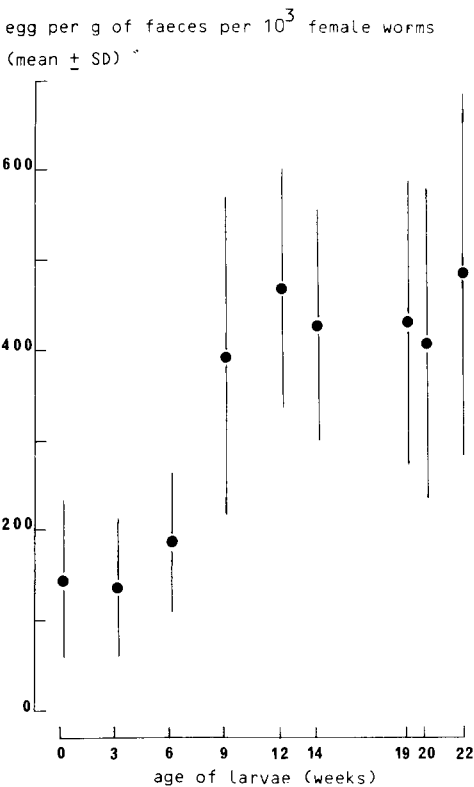


Fig. 3. — Increase in egg production of female worms when time of storage of infective larvae increases.

are, to a large extent, similar to those observed by other authors (Ciordia *et al.*, 1966; Kerbœuf, 1978a; Mallet and Kerbœuf, 1984). However, a comparison of the results showed that some differences did exist, especially in relation to the rapidity of the decrease in infectivity and the occurrence of a maturation period.

The rapidity of the decrease in infectivity seemed highly variable from one species of parasite to another and also as a function of experimental conditions. Ciordia *et al.* (1966) obtained a more rapid decrease (from 53.3% to 37.7%) than in the present work, in worm burdens of adult *T. colubriformis* established from third-stage larvae after storage for three weeks at 25 °C and using rabbits and guinea-pigs to assess infectivity. In the present experiment, such a decrease occurred only after nine weeks of storage at 24 °C but the animals used for the experiment were lambs.

A maturation period was observed with *Heligmosomoides polygyrus*. It has been shown that infectivity reached a maximum after three weeks of larval storage at 22 °C (Kerbœuf, 1978a; Mallet and Kerbœuf, 1984).

With *Trichostrongylus retortaeformis*, a rabbit parasite, a maturation period was observed after two to four weeks of storage at 20 °C including a four-day faecal culture at the same temperature (Ford, 1971).

It seems that the length of the maturation period varied according to the species and conditions of egg development. A lower temperature would increase the time until maximum infectivity was reached whereas a higher temperature would decrease it so that it became imperceptible. For example, no maturation period could be observed in calves infected with *Ostertagia ostertagi* and *Cooperia oncophora* larvae collected after a 7-day faecal culture and stored for 37 days at room temperature (Smith, 1976).

Two hypotheses can explain why third-stage larvae were, as early as the beginning of the experiment, at their maximum infectivity. The first is that the faeces were incubated at 24 °C which is the optimum temperature for egg development and third-stage larvae were stored at the same temperature. If a maturation period did exist, it was certainly shorter than ten days in these conditions. The second is that the larvae were collected with a Baermann apparatus which might possibly have selected the more motile part of the larval population.

Fecundity of female worms

An increase in egg production of adult worms established from larvae with reduced infectivity has been observed previously with *H. polygyrus* (Kerbœuf, 1978b). These observations are confirmed by the present results obtained with *T.*

colubriformis. Larvae stored for at least nine weeks at 24 °C developed into adult worms, egg production of which was twice that of worms developed from fresh larvae. These results do not correspond with those of previous studies. Two different opinions have often been discussed: for *Ostertagia ostertagi*, Michel (1967, 1969) described a regulation phenomenon which limits the egg production of the whole population of worms to a constant number of eggs whatever the number of female worms. Other workers (Kingsbury, 1965; Besubick *et al.*, 1970; Stampa and Linde, 1972; Gibson and Parfitt, 1975; Kerbœuf, 1982) thought that a relationship exists between the total egg production and the size of the worm population for different strongyle species. If the regulation phenomenon observed by Michel did occur in the present experiment, the total egg production (egg per gramme of faeces) would have been the same for each batch of animals and in fact, it doubled between the 6th and the 9th week of larval ageing. These results were also in contradiction with the second opinion because total egg production increased while worm population decreased. Thus, these results can be explained only by another factor in relation to the ageing of infective-larvae. This phenomenon is of epidemiological

importance when attempting to assess the importance of fluctuations in pasture contamination which depend on survival of larvae. As far as *Trichostrongylus colubriformis* is concerned, field studies (Boag and Thomas, 1970; Gibson and Everett, 1967) have shown that infective larvae survived in great numbers on pasture (up to 20 weeks) in winter. More generally, the part played by overwintering larvae in renewing the contamination of pastures in spring has often been discussed (Rose, 1961, 1963a, b) but that of larvae surviving over summer has been rarely mentioned. However it may be of some importance in the rise of infection often observed in autumn.

Climatic conditions during July and August may sometimes stop egg development (Gruner *et al.*, 1980). Thus, in autumn, contamination could arise from larvae developed in June. These larvae, as shown by the present paper, can survive up to 22 weeks *in vitro* at 24 °C and, after nine weeks of storage, the egg production of adult females increases thereby compensating for the loss of infectivity.

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Summary

Relationships between egg production and infectivity were studied with infective larvae of *Trichostrongylus colubriformis* which had been stored for up to 22 weeks at 24 °C. After different storage times, larvae were administered to lambs and the number of parasites which reached the adult stage was counted five weeks after infection. Egg production was estimated for the same batches of animals by performing a quantitative coproscopical examination from the third week of infection onwards. Infectivity was maximum and constant during the first six weeks of storage and then decreased regularly from the 9th week to the end of the experiment. However, the number of eggs produced per female worm was constant during the first 6 weeks then doubled and remained constant from the 9th to the 22nd week of storage.

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