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THE DURATION OF PRE-PARASITIC DEVELOPMENT OF TRICHOSTRONGYLUS VITRINUS AT CONSTANT TEMPERATURES

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Summary: *The pre-parasitic development of* Trichostrongylus vitrinus *was studied at a range of constant temperatures from* 24 *to* 4.6 °C. *There was no evidence either of a discontinuity at the lower end of the time and temperatures curve or of excessive mortality at the low temperatures. The apparent difficulty of relating this finding to the established epidemiological model is discussed briefly.*

Trichostrongylus vitrinus'un 24–4.6°C'ler arasında değişik ısı derecelerinde pre-parazitik gelişme süresi.

Özet: 24–4.6 °C *arasında değişen ısılarda* Trichostrongylus vitrinus'*un pre-parazitik gelişmesi üzerinde çalışıldı. Parazit yumurtalarında gelişimin oldukça düşük ısılarda da devam ettiği görüldü. Ayrıca düşük ısılarda fazla bir mortalite gözlenmedi. Bu bulguların epidemiyolojik modelle olan ilişkisi kısaca tartışıldı.*

Introduction

The changes in the composition of the trichostrongylid worm burden of lambs at pasture during the course of the grazing season have been studied in Great Britain by Heath and Michel(3), Cornwell(2) and Boag and Thomas(1). These observers all found that substantial burdens of intestinal Trichostrongylus only appeared later in the year than those of Ostertagia. In 2 of the 3 years of observations made by Cornwell (2) and in those made by Boag and Tho-

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mas(1) *T. vitrinus* was the predominant species. Boag and Thomas(1), suggested that the succession of species could be explained in terms of the lenght of free living development of the individual species. This paper records measurements of the time required for completion of development by *T. vitrinus* from egg to infective larvae (L_3) at constant temperatures in the laboratory.

Materials and Methods

Faeces were collected from the rectum of a sheep with a monospecific infection of *T. vitrinus* and were set up in their experimental environment within one hour of collection.

Developing material maintained by method A (see below) was incubated in small insulated water baths housed in a cold room. These baths ran at 4.6, 6.7, 10.2, 12.1, 14.8, 18.5 and 20.6 °C and the extremes of variation were ∓ 0.2 °C. Materials were also incubated at 24 ∓ 1 °C in a climate room. The material maintained by method B was incubated at 5.3 and 7.6 °C in commercial cooled incubators with a fluctuation of ∓ 1 °C and in a laboratory oven at 9.3 °C sited in the cold room: extremes of variation in the oven were ∓ 0.1 °C.

Two methods, A and B, were used and are illustrated in Fig. 1. Method A: 8–10 g of faecal pellets were placed directly in each 150 × 25 mm test tube and the tube was plugged with cotton wool. The plug was moistened daily a few drops of water. Six tubes were set up at each temperature. For examination an emulsion of 3 pellets was prepared in tap water, filtered through a sieve of 0.7 mm aperture and centrifuged briefly, the sediment was washed 3 times and then transferred to a petri dish and searched at a magnification of X 20. The first 30 larvae seen were collected with a capillary pipette and tested with NaOCl. Method B: when the faeces were collected from the sheep a 10% emulsion was prepared in tap water and mixed with absorbent mineral granules (cat litter) to give a crumbly mixture. An aliquot of the mixture containing approximately 0.7 g of the original faecal material was wrapped in hemp paper and suspended in a tube as shown in Fig. 1. The tube was closed with polythene film. For examination, 10 ml of water was added to the tube and the packet was pushed down into the water. After 2 hr at room temperature to allow migration of the larvae, the packet was discarded, the larwae were counted and tested with NaOCl.

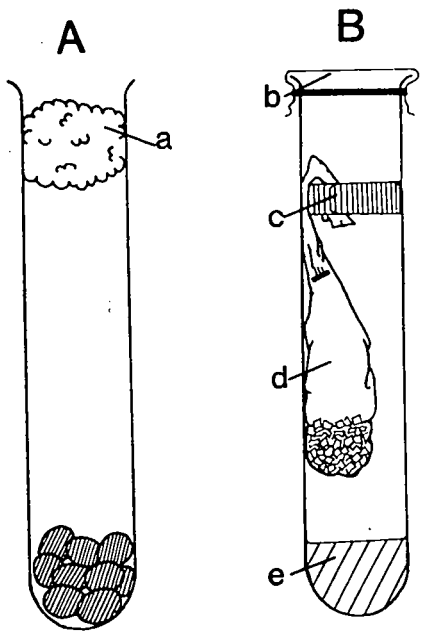

Fig. 1. Two methods (A and B) of maintaining developing eggs and larvae. Cotton wool plug (a), polythene film (b), plastic split ring (c), hemp paper sack (d), 2 ml tap water (e).

Larvae were held in 0.2 % NaOCl for 30 minutes and then killed by addition helminthological iodine to a finished concentration of approximately 0.2 % iodine. The stained larvae were examined at X 100 and classified as L_3 (those which had esheathed) or not L_3.

Samples of faeces were examined at intervals of 2 or 3 days starting at times suggestedly knowledge of the time and temperature curve for *Ostertagia circumcincta*. The times recorded here are those by which $> 50\%$ and $> 85\%$ (Method A) or $> 85\%$ (Method B) of larvae were classified as L_3 by the NaOCl test.

Results

The results are shown in Fig. 2. Development in whole pellets, as studied by method A, was successfully completed to L_3 at all temperatures investigated. More than 85% of the larvae recovered had reached the infective stage at times ranging from 6 days at 24° to 63

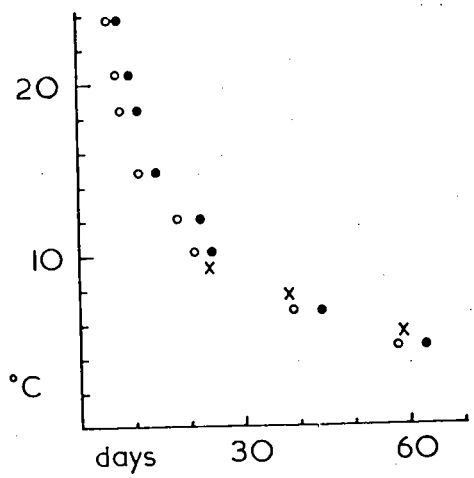

Fig. 2. Time required by *T. vitrinus* for development to L3 at constant temperatures shown: method A 50% (o), 85% (.); method B 85% (x).

days at 4.6°C. The ability to complete development at temperatures as low 5.3° was confirmed by method B. The mean yields of L_3 calculated as percentages of the estimated numbers of eggs set up for study by method B at 5.3, 7.6 and 9.3°C were 74, 94 and 81%, respectively.

Discussion

Two methods of recovering larvae for examination were used. The laborious method A, in which recovery does not depend on the motility of the larvae, was used in anticipation of the possibility that while eggs would hatch at the lower temperatures, the emerging larvae might die as L_1 or L_2. In fact method A established the ability of some larvae to complete development to L_3 at all the temperatures tested and the existence of a regular relationship between temperature and development time, but the method did not give an estimate of the propotion of eggs which reached L_3. Method B showed that a high propotion of eggs completed development at temperatures down to 5.3°C.

The limited amount of published data indicates that other species of the genus Trichostrongylus do not develop at such low temperatures as *T. vitrinus*. Levine and Anderson (4), found that *T. colubri-*

formis would not usually develop to L_3 when soil surface temperatures were below 10°C. Mirzayans(5), demonstrated that at 5°C. the eggs of *T. axei* fail to develop to the infective stage.

It is not immediately obvious how our laboratory information on the rate of development to L_3 can be related to the observed epidemiology of *T. vitrinus*. Boag and Thomas(1), who reported a sudden rise of *T. vitrinus* burdens to quite substantial numbers in September, concluded that there was only one generation of *T. vitrinus* during their trial and that this species was transmitted by the ewe egg output. Using the development times taken from our Fig. 2 and assuming mean monthly temperatures of 9°C. in May, 12°C. in June and 14°C. in July, the period from the time of the post parturient rise in ewe egg output to the end of july appears to offer four times the number of degree days necessary for the completion of development of *T. vitrinus* to the infective stage. While it is possible to devise a number of hypothetical explanations for the 'lost time' of more-than 60 days in terms of the time taken for larvae to return from the soil or mat to a location in which they can be ingested by lambs, or in terms of changes in the chances of ingestion resulting from changes of eating habit as the pasture gets eatden down at the end of the summer, it is difficult to see why the epidemiology of Ostertagia would not be influenced in the same way.

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