The distribution of larval *Aspiculuris tetraptera* Schulz during a primary infection in *Mus musculus*, *Rattus norvegicus* and *Apodemus sylvaticus*

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SUMMARY

The larvae of *Aspiculuris tetraptera* were found in the mid-colon of mice within hours of infection. When the colon was divided into 10 equal sections the larvae were mainly found in sections 5, 6 and 7 during the first 6 days of the infection. The worms entered the crypts of Lieberkühn in this region on day 1 and remained there until day 4 or 5. After this time they left the crypts and returned to the lumen of the colon. On day 7 the worms emigrated anteriorly and thereafter were recovered only from the proximal region of the colon (sections 1-4), although in heavier infections a few larvae remained in the mid-colon.

The initial establishment site was the same in both *Rattus norvegicus* and in *Apodemus sylvaticus*, but an infection with *A. tetraptera* in the abnormal hosts, *R. norvegicus* and *A. sylvaticus*, was characterized by less than 7% of the inoculum becoming established, a slower rate of growth and a wider distribution centering around the preferred site. The small number of established larvae was lost from the rat before day 12 and from *A. sylvaticus* before day 8, whereas larvae persisted in laboratory mice for a longer period.

INTRODUCTION

The oxyurid nematode *A. tetraptera* has a direct life-cycle in which eggs, voided with the host faeces, take about a week to reach maximum infectivity at 24 °C (Anyá, 1966*a*). Larvae escape from eggs, after ingestion by the host, in the lower intestine and establish in the colon, where the infection becomes patent on day 24 (Anyá, 1966*b*). Although several previous authors have studied *A. tetraptera* (Philpot, 1924; Hsu, 1952; Chan, 1955; Fisher, 1958; Anyá, 1966*a, b*) little is known about the distribution of the larvae in the colon during the course of a primary infection. This paper presents the results of a study in which the distribution of the larvae during a primary infection was examined in the usual host, *Mus musculus* and in the abnormal hosts, *Rattus norvegicus* and *Apodemus sylvaticus*.

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MATERIAL AND METHODS

Laboratory mice

Three strains of mice were used in this work. Specific pathogen free (SPF), CFLP strain mice were obtained from Carworth Europe Ltd. A small randomly breeding colony of mice (originally CFLP) was maintained in the laboratory; these animals being referred to as CFLPb mice. Naturally infected TF1 mice (Tuck and Sons Ltd.) were killed to provide worms from which eggs were obtained for the preparation of infective material. Mice were housed in plastic cages (North Kent Plastic Cages Ltd) on sawdust litter in numbers not exceeding 12 mice per cage and pellet food (Diet 41B, Oxoid) and water were supplied *ad libitum*.

Rattus norvegicus

Hooded Wistar rats were obtained from a small colony of randomly breeding rats maintained at Bedford College, University of London. Rats were kept in pairs on sawdust litter in plastic cages and pellet food and water were supplied *ad libitum*. To prevent the experimental animals from becoming naturally infected with *A. tetraptera* all cages were cleaned daily.

Apodemus sylvaticus

The trapping, handling and maintenance of wild *A. sylvaticus* has been described previously (Behnke & Wakelin, 1973). The population from which the experimental field mice were taken was not infected with *A. tetraptera*; naturally occurring *Syphacia stroma* were removed by treatment of the host with piperazine citrate (Behnke & Wakelin, 1973).

Aspiculuris tetraptera

In order to infect the experimental animals, eggs of *A. tetraptera* were obtained by crushing gravid female worms on a metal grid. The liberated eggs were incubated in distilled water in Petri dishes at 24 °C for 1 week. At the end of this time, the eggs were transferred to a small conical flask and the concentration of embryonated eggs was adjusted to give the required dose in 0.1 ml of suspension. A magnetic stirrer was used to ensure an even distribution of eggs in the suspension and egg counts on sample doses showed that the number of eggs in subsequent doses varied by no more than 10%. The inoculum was given orally to the mice through a blunted hypodermic needle.

Recovery of worms from infected animals

Animals were killed, either by means of chloroform or by cervical dislocation, and the caecum, colon and rectum were removed immediately after death. In one experiment the colon was ligatured in five places, about 2 cm apart, to prevent the movement of gut contents, and then stored in Petri dishes at -18 °C until examination. On thawing the colon was divided into 10 equal sections, each of which was opened under water in a separate Petri dish and scraped clean with the

edge of a glass slide. Each dish was then examined under a binocular microscope and larvae were removed and counted.

In other experiments, a different procedure was adopted for worm recovery. The colon was immediately removed and divided into sections, as in the previous experiment. Each section was opened and incubated at 37–38 °C in a separate Petri dish of Hanks' saline to encourage the worms to emerge from the mucosa. Larvae were counted as they were removed with a pipette.

Histological studies

Sixteen male mice were infected with 500 eggs and then four were killed on each of the following occasions: 24, 48, 96 and 168 h after infection. The colon was fixed in Bouin's fixative for 24 h, dehydrated in ethanol, cleared in toluene, embedded in paraffin wax and sectioned at 6–8 μ m. The sections were then stained in haematoxylin and eosin.

RESULTS

The distribution of larvae following an infection of 250 eggs in M. musculus

In the first experiment 25, 4-week-old CFLP male mice were each given 250 embryonated eggs. Two mice were removed from the group every 12 h for 1 week following infection and the remainder was killed on day 10. The results of this experiment are presented in Fig. 1.

The larval worms were confined almost exclusively to sections 5 and 6 of the colon during the first six days of the infection. As early as 12 h post-infection 20 worms (44 % of the mean worm burden for days 1–7) were recovered from this region. No worms were found in the caecum at any time. After the sixth day, the worms began to move anteriorly and by day 7 this emigration was clearly evident. On day 10 the worms were found in the first four sections of the colon and it was from this region that the mature worms were recovered 4 weeks after infection.

The distribution of larvae following an infection of 1000 eggs in M. musculus

Twelve, 4-week-old male CFLPb mice were given 1000 infective eggs of *A. tetraptera*. The times at which individual mice were killed were as follows; 24 h (1 mouse), 48, 72, 96 h (2 mice on each occasion) 120, 168, 192, 216 and 240 h (1 mouse on each occasion). The distribution and the mean worm recovery on specific days after infection are shown in Fig. 2. In this infection the sequence of events appeared to be very similar to that in the previous experiment. Thus here also the mid-colon (sections 5–7) was the preferred region. It can be noted that whereas in the first experiment larvae were seldom found in more than two adjoining sections between days 1–5, they seemed to be more widely distributed in the heavier infection and were frequently recovered from three or four sections, although the maximum number was always in section 5, 6 or 7. No mouse was killed on day 6, but it is clear that on day 7 the worms were moving anteriorly and the majority were recovered from the proximal region (sections 1–4) on day 8. There was no further change in distribution on either day 9 or 10, but it was observed that a small number of larvae still remained in the mid-colon and distal colon (sections 8–10) on day 10.

Fig. 1. Exp. 1. The distribution of the larvae of *Aspiculuris tetraptera* in the mouse colon after infection with 250 eggs.

A histological examination of the infected colon

Examination of the histological material showed that the larvae were found in the lumen of the crypts of Lieberkühn for at least the first 4 days of the infection, sometimes persisting in this location until day 7 (Pl. 2B). The anterior end of the larvae penetrated deeper and by 96 h after infection could usually be seen at the base of the crypt, where the epithelial cells were compressed and flattened (Pls. 1B; 2A). As early as 48 h after infection, crypts containing larvae appeared to contain

Fig. 2. Exp. 2. The distribution of the larvae of *Aspiculuris tetraptera* in the mouse colon after infection with 1000 eggs.

epithelial and goblet cells which were smaller and contained less material than those in the non-infected neighbouring crypts (Pl. 1 A, B, C). There was, however, no substantial damage and larvae were not seen completely in the lamina propria. As in Exps. 1 and 2 all the worms examined between days 0–7 were in the mid-colon. It was only on day 7 that worms were seen in the lumen of the colon in large numbers (Pl. 2 E) but some still persisted in the crypts (Pl. 2 B).

Table 1. *Exp. 4. The recovery and growth of the larvae of Aspicularis tetraptera from Rattus norvegicus and CFLPb strain laboratory mice after infection with 1000 eggs (1 animal killed on each day)*

Days post-infection	<i>R. norvegicus</i>				<i>M. musculus</i>			
	No. larvae recovered	No. larvae measured	Length (μ m)		No. larvae recovered	No. larvae measured	Length (μ m)	
			Mean	S.D.			Mean	S.D.
3	65	39	218.3*	32.6	173	59	239.9*	23.6
4	3	Not measured			137	Not measured		
5	8	8	346.9**	38.9	202	30	367.1**	32.6
9	11	9	582.0***	47.8	357	54	750.0***	64.3
12	0	—	—	—	83	53	835.5	99.3

Statistical analysis of results by Student's *t*-test; **P* < 0.001, ** *P* = Not significant, *** *P* < 0.001.

The distribution of larvae in rats

Six hooded Wistar rats (one male and five female) and six male CFLPb control mice were infected with 1000 eggs of *A. tetraptera*. One animal from each group was killed on days 3, 4, 5, 9, 12 and 17. The larvae were recovered, counted and left overnight at 4 °C in Hanks' saline before being measured by camera lucida. All the measurements were completed within 24 h of the retrieval of the worms.

Three criteria were used in order to assess the suitability of the rat as a host for *A. tetraptera*; the number of worms present, the distribution of the worms in the colon, and the size of the recovered worms. The results (Table 1; Fig. 3) show that the infection in the rat differed from that in the mouse in all three points. The rat had fewer worms on all the occasions compared, the worms in the rat also being smaller on day 3 (*P* < 0.001) and on day 9 (*P* < 0.001). No larvae were found in the rats killed on days 12 and 17. Fig. 3 shows that in spite of the lower worm recovery from rats, the established worms were found in the mid and distal colon of this host on days 3, 4 and 5. On day 9, however, the larvae were anterior to those found on days 3–5, but the emigration was not completed at this time, as in the control mouse.

The distribution of larvae in Apodemus sylvaticus

Since *A. sylvaticus* is known to be a host of *A. tetraptera* (Hall, 1916; Bernard, 1963), it was of interest to discover whether *A. tetraptera* would show the site specificity and an emigration pattern like that of infections in the laboratory mouse.

Seven *A. sylvaticus* (four male and three female) and eight male CFLPb control mice were given 1000 eggs of *A. tetraptera*. The mice were killed on the days shown in Table 2 and the larvae were recovered by incubation in Hanks' saline. They were counted and measured as already described.

A. tetraptera was found to be unable to become established as well in *A. sylvaticus* as it was in mice. Twenty-four hours after infection and on day 3 fewer larvae were

Fig. 3. Exp. 4. The distribution of the larvae of *Aspiculuris tetraptera* in the colon of *Rattus norvegicus* and CFLPb strain laboratory mice after infection with 1000 eggs.

recovered from *A. sylvaticus* than from the control mice. By day 4 even fewer larvae remained in *A. sylvaticus*. The growth of worms proceeded at a slower rate but their distribution showed that they became established in a region of the colon corresponding to the preferred site in control mice. The larvae were, however, more widely distributed and whereas on day 1 they were confined to four sections in the mouse (sections 4-7), they were spread over 6 sections in *A. sylvaticus* (sections 5-10). Not only was there a maximum number in the usual region (sections 5-7) but a second peak was observed in section 10, indicating that larvae were being lost from the first days of the infection. This was confirmed by the lower worm burdens on day 4 and the absence of worms on days 8, 10, 14 and 16.

Fig. 4. Exp. 5. The distribution of the larvae of *Aspiculuris tetraptera* in the colon of *A. sylvaticus* and CFLPb strain laboratory mice after infection with 1000 eggs.

DISCUSSION

Chan (1955) found that the larvae of *A. tetraptera* became established in the mid-colon soon after infection and began to move towards the distal colon on day 4 before emigrating anteriorly towards the proximal colon. Fisher (1958) could not confirm this emigration pattern and Anya (1966b), reviewing all the major previous studies on the biology of *A. tetraptera*, suggested that the emigration reported by

Table 2. *Exp. 5. The recovery and the growth of the larvae of Aspiculuris tetraptera from Apodemus sylvaticus and CFLPb strain laboratory mice after infection with 1000 eggs (1 animal killed on each day)*

Days post-infection	<i>A. sylvaticus</i>				<i>M. musculus</i>			
	No. larvae recovered	No. larvae measured	Length (μm)		No. larvae recovered	No. larvae measured	Length (μm)	
			Mean	S.D.			Mean	S.D.
1	25	19	173.7*	31.7	218	37	185.8*	35.6
3	32	31	295.2**	25.3	187	48	318.2**	37
4	9	9	344.4***	24.3	113	39	370.5***	24.3
8	0	—	—	—	305	59	742.0	34.6
10	0	—	—	—	254	Not measured		

Statistical analysis of results by Student's *t*-test: **P* < 0.1, ***P* < 0.01, ****P* < 0.01.

Chan resulted from the passage of non-viable larvae with the faeces, whilst the larvae destined to reach maturity were in the crypts in the colon.

The present study differed from that of the previous authors in a number of respects. The distribution of the larvae in the colon was recorded in more detail than in other studies by a technique designed to simplify the task of deciding whether or not a movement of the population of worms had taken place.

Chan based his conclusions on a consideration of the pooled totals of worms recovered from each of three regions on the relevant day. The distribution in individual mice, however, showed that on day 4, two out of four, and on day 6, three out of five mice, had greater numbers of larvae in the mid-colon. It could be that by dividing the colon into three sections, Chan had bisected that region of the colon identified in the present study as the preferred site for the worms (approximately 60% along the length of the colon). Anya pooled all similar sections of the colon from the mice killed on a particular day, before examining them for worms. This procedure would have disguised any individual variation between the mice and it is possible that Anya's technique would not reveal any local concentration of worms. The present work has shown that during the first week of infection the majority of larvae could be recovered from the mid-colon. In some mice the concentration appeared to be more pronounced than in others, but the region of maximum occurrence corresponded normally to section 5, 6 or 7. In infections consisting of about 60 larvae, most of the worms could be found in two adjoining sections, but a wider distribution (as many as four sections) prevailed in infections where the mean worm burden was about 150 worms.

Mya (1955), Fisher (1958) and Anya (1966*b*) recorded that the larvae of *A. tetraptera* entered the crypts of Lieberkühn soon after infection, but to-date no photographs have been published to illustrate these findings. The present results confirm and expand on the observations made by the previous authors. Thus, here the larvae were only recorded in the mid-colon during the first week of infection and it was in this location that the crypts were occupied. Evidence of superficial damage to host tissues was presented as manifest by the compressed epithelial

cells adjacent to larvae and perhaps local distortion of cell function, such as a lowered mucus output by goblet cells.

A. tetraptera has been reported from wild populations of both *Rattus norvegicus* and *Apodemus sylvaticus* (Bernard, 1963). In addition Mathies (1959) found that the albino laboratory rat was an inferior host to the laboratory mouse for this parasite. With the discovery, in the present work, of a very precise initial establishment site for the larvae of *A. tetraptera* in the mouse colon, it was pertinent to expand this observation to other potential hosts. Both abnormal hosts were found to be susceptible to infection and further, the established worms showed signs of a site specificity similar to that in CFLP mice. In all the animals autopsied, the worms were recovered from the mid-colon or even distal to it, there being no larvae in the anterior colon. The site specificity observed in abnormal hosts differed from that in the mouse, there being a greater range of sections from which the parasite could be recovered. In the field mouse, the small population of established worms appeared to be gradually displaced distally, few larvae remaining in the colon after day 4. The larvae were more successful in the rat, however, where the worms recovered on day 9 were anterior to the initial establishment site, suggesting that an emigration may have been undertaken in this host, but it lagged behind events in CFLP mice, where the emigration was already completed at this time. In neither the rat nor in *A. sylvaticus* did *A. tetraptera* grow as quickly as in CFLP mice.

The loss of the larvae which have successfully established is explicable in several ways. First, it is possible that the worms, being incapable of deriving nutrients from the abnormal environment, survived until stored nutrients were used up. This is unlikely, however, since the larvae in both abnormal hosts achieved over a twofold increase in length before their eventual elimination. Further, it is known that larvae can survive longer in hydrocortisone treated *A. sylvaticus* (Behnke, unpublished). Secondly, it must be considered that a host response caused the loss of larvae as is known to be the case in *Nematospiroides dubius* (Cross, 1960; Cross & Duffy, 1963) and *Nippostrongylus brasiliensis* (Parker, 1961). It is possible that a local inflammatory response in the mid-colon brought about an adverse situation, from which the larvae escaped by an anterior emigration. Such a response might be expected to occur earlier in the abnormal host, perhaps forcing the larvae into the gut lumen before they were sufficiently developed and prepared for such an emigration, thus causing the loss of all the worms in this situation.

In the rat, it is suggested that events proceeded in a slightly different way. The survival of the larvae in rats for 9 days implies that the worms were not forced into an early emigration, but the observation that such an emigration was attempted suggests that the worms were unable to complete it, most probably because of an inability to maintain a position in the lumen of the rat intestine.

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KEY TO THE LETTERING OF THE PLATES AND FIGURES

a.l. anterior end of larva l. larva M.w.r. Mean worm recovery

EXPLANATION OF PLATES

PLATE 1

- A. Longitudinal section through the mid-colon 24 h after infection showing a larva entering a crypt of Lieberkühn. ($\times 150$.)
- B. Transverse section through a 48 h-larva in the lumen of a crypt of Lieberkühn. The large, mucus-secreting goblet cells seen in neighbouring crypts appear to be smaller in the infected crypt. ($\times 600$.)
- C. Longitudinal section through a crypt of Lieberkühn 48 h after infection showing the intimate contact between the larval cuticle and the host epithelial cells. ($\times 600$.)
- D. Transverse section through the mid-colon 96 h after infection showing the anterior end of a larva deep in the crypt lumen. ($\times 150$.)

PLATE 2

A. Transverse section through the colon 96 h after infection, showing the anterior end of a larva in the crypt lumen. ($\times 300$.)

B. Transverse section through a 7-day larva in the lumen of a crypt of Lieberkühn. ($\times 300$.)

C, D. Two successive sections through a crypt 96 h after infection, showing a larva pushing its anterior end against the wall of the crypt. In C the anterior end has penetrated into the lamina propria. ($\times 300$.)

E. Seven-day larvae in the mid-colon moving out of the crypts into the gut lumen before the anterior emigration. ($\times 150$.)

F. Transverse section through a 7-day larva lying in one of the colonic rugae in the mid-colon. This worm has penetrated below the surface layer of epithelial cells and the cuticle is in direct contact with the lamina propria. ($\times 300$.)