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The number and distribution of *Brugia pahangi* in cats at different times after a primary infection

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Summary

The number of larvae and adults of *Brugia pahangi* and their distribution throughout the lymphatics and extra-lymphatic tissue were studied in cats infected by subcutaneous injection of larvae into their hind feet. For the first 20 days approximately 55% of the inoculum is recovered as living worms. After 25 days the recovery falls by a half. It is suggested that this loss of worms may be due to either the developing immunological response or the moult from the 4th to the 5th stage. Larvae penetrate the lymphatics rapidly (50% within 3 h) and migrate to the popliteal lymph node after about 20 days they migrate back down into the afferent lymphatic.

Key words: *Brugia pahangi;* cat; adult worm recovery; larval development.

Introduction

Whilst most nematode parasites of the intestinal tract of rodents are expelled by an immune response about 14 days after infection the filarial worms, be they of rodents or other mammals, are usually very long lived. For example *Brugia pahangi* survives for several years after infective larvae have been inoculated on a single occasion into cats (Wilson and Ramachandran, 1971; Denham et al., 1972). Most cats become microfilaraemic after subcutaneous injection of third stage larvae of *B. pahangi.* A recent check of our records revealed that of 359 cats eligible for observation 96.1% became microfilaraemic after injection of infective larvae, 3.9% remained amicrofilaraemic and of the microfilaraemic cats 2.2% became spontaneously amicrofilaraemic. Those cats which do not become microfilaraemic after a single inoculation of larvae fail to become

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microfilaraemic after subsequent challenges which suggests that they are inately resistant (unpublished observations).

Ewert (1971) showed that if cats are infected with larvae of *Brugia malayi* into the hind feet on a single occasion the larvae migrate to the popliteal lymph nodes and then at about the time of the ecdysis from the fourth to fifth life-cycle stage, they migrate back into the lymphatics afferent to the popliteal nodes.

In the present paper the survival of *B. pahangi* is determined in cats from two hours to one year after the inoculation of infective larvae.

Materials and Methods

The methods for producing infective larvae in *Aedes aegypti* and collecting them in a Baermann apparatus were described by Denham et al. (1972). Carefully selected, active and undamaged, infective larvae were counted into batches of 100, collected into 1 ml syringes and inoculated subcutaneously into the dorsal surface of both hind feet. The syringes were then washed out and any larvae found in the washings counted to determine precisely how many larvae had been inoculated.

The procedure for post mortem examination was similar to that of Denham et al. (1972) but, to recover the small larvae expected soon after infection, the lymphatics and lymph nodes were teased with fine forceps and mounted needles under dissecting microscopes. The lymphatics and nodes of the hind limbs, pelvic region, tail and para-aortic system were studied separately. When the active search for larvae had become unproductive the lymphatic tissues, the detached limbs, pelvic region, hind body, tail and skin were soaked overnight in saline and the fluid examined for worms the next morning. A record was kept of the number of worms found, and the site from which they were recovered.

Results

The distribution of worms between different sites is indicated in Fig. 1. The distribution is shown as the percentage of the larvae found in 1. the soakings of the skin and hind limbs, 2. the lymphatic afferent to the popliteal lymph node, and 3. the popliteal lymph node. A few larvae were found in other sites such as the efferent lymphatic from the popliteal lymph node and the lymphatics of the tail but their numbers were so small that they are excluded from this histogram.

The results for the different times after autopsy will be considered separately.

The first 48 h after infection. 3 cats were autopsied at 3 h, 3 at 6 h, 4 at 12 h, 5 at 24 h and 5 at 48 h after infection. A mean of 55.7% (SD 8.5) of the larvae inoculated into these cats was recovered at autopsy. The distribution of the larvae between the three anatomical sites indicated above was the same at 3 and 6 h after infection. The largest percentage of worms was found in the fluid in which the skin and hind limbs had been soaked showing that the worms had not yet penetrated the lymphatics. At 12, 24 and 48 h the large majority of the larvae were in the node, very few were found in the soakings and a few in the afferent lymphatic.

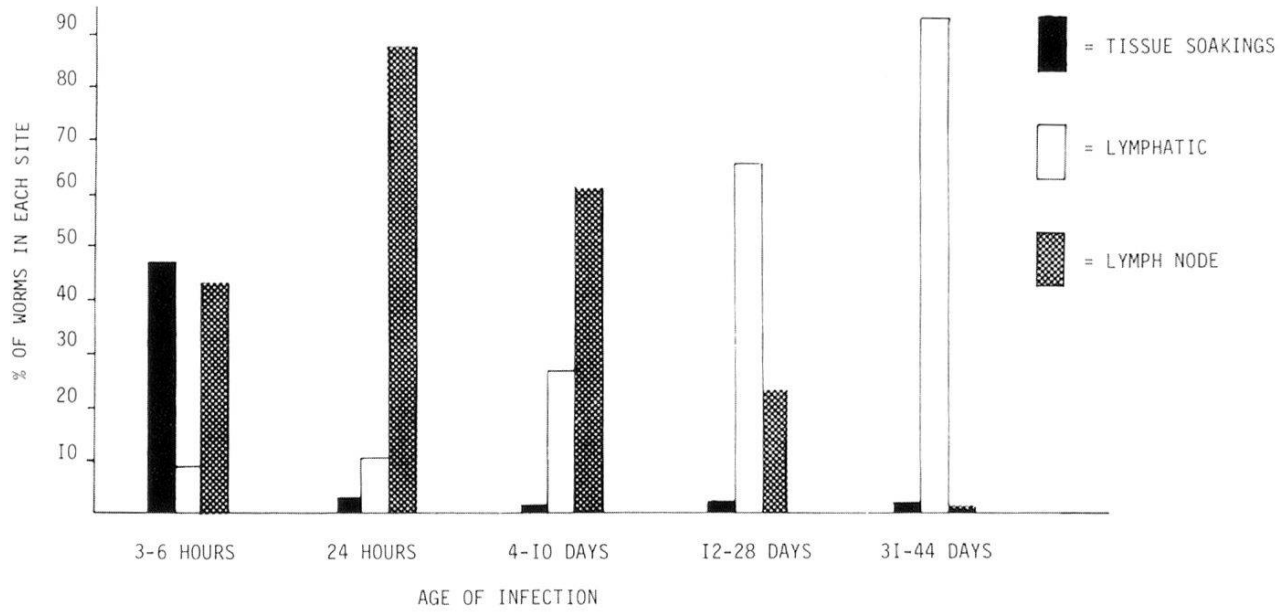

Fig. 1. A summary of the anatomical distribution of *Brugia pahangi* during the first 44 days of infection demonstrating the migration of larvae into the lymphatic then the lymph node and finally back down into the lymphatic.

Histologically the larvae at this stage appear to be in the perinodal sinus and this probably explains why in occasional animals many larvae were found in the afferent lymphatic. Whilst we attempted to separate the node and lymphatic at the point where they joined the perinodal sinus is sometimes extended as a bulb which harboured many of the worms which were however credited by us to the afferent lymphatic.

4–20 days after infection. The mean recovery from 17 cats in this period was 56.5% (SD 8.4) of the number of larvae inoculated which compares well with the 55.7% recovered in the first 48 h. It is clear that there is no decline in numbers until at least 20 days after infection. During this period there was a diversity in the distribution of the larvae between the popliteal node and its afferent lymphatic with a marked tendency for the cats with older infections to have the majority of their larvae in the afferent lymphatic.

24–42 days after infection. The mean recovery from the 13 cats in this period was 43.4% (SD 13.9). During this period the large majority of the worms was recovered from the lymphatic afferent to the popliteal node.

73 days to one year post infection. The mean recovery from the 22 cats in this group was 28% (SD 7.6). This is significantly less than the 56% recovery in the period up to 20 days after infection although as in the earlier groups the SD represented 8.9 of the mean ($p = 0.04$).

Table 1. Worm recoveries between days 12 and 42 from individual cats as a percentage of the number of the larvae inoculated

Days	Individual % recoveries	Days	Individual % recoveries
12	52	32	34
14	50, 57	35	35
19	52, 54, 64	36	63
20	67, 67, 77	37	49
24	69	38	44, 55
25	37, 50	39	35
28	29	42	19
31	46		

Discussion

The recoveries of larvae during the first 20 days are very consistent and it is clear that very few if any of the worms die after they reach the lymphatics during this period although 40% of the inoculum was not recovered. Schacher (1962) found that third stage larvae moulted to the fourth stage on days 8 or 9 after infection which suggests that neither the third larval stage nor the moulting larvae are susceptible to the host's defence mechanisms during a primary infection. This is in sharp contrast to what happens in cats which have been vaccinated with irradiated larvae of *B. pahangi* (Oothuman et al., 1979). In these partially immune animals the principle attack upon the challenge infections occurred during the first day of infection and once past this stage there was little death of the challenge larvae.

In contrast to the cats killed in the first 20 days of the infection, when there was a recovery of 56%, only 28% of the inoculum was recovered from those cats killed between 73 days and 12 months after infection. Thus approximately 50% of the worms which successfully invaded the lymphatics and almost completed their development died after 20 days. Unfortunately the variability in the percentage worm recoveries increased markedly after 20 days so that it is difficult to decide precisely when this destruction of half the worm population occurred but it appears to start from 25 days after infection. Table 1 shows the recoveries of worms from individual cats between 12 and 42 days. It can be seen that before day 25 none of the 10 cats yielded less than 50% of the larvae inoculated whereas from 25 days onwards 8 of 12 cats yielded less than 50%. Schacher (1962) has shown that male and female *B. pahangi* undergo their final moult about 23 days and 27–33 days after infection respectively. It is possible, therefore, that in primary infections the moult from fourth to fifth stage or the appearance of fifth stage worms stimulates the host to kill many of the worms. Alternatively it is possible that this temporal relationship is coincidental and that the death of the worms is due to a host response which takes 25 or more days to develop in different cats.

One way of determining whether the decline in numbers of worms which survive this period of apparent danger is due to an immune response or not would be to immunosuppress cats from the time of infection and to compare worm recoveries from these cats with that from untreated controls. It is our intention to investigate this possibility.

If the percentage of parasites which die about 25 days after infection can be increased by stimulation of the immune response this would be of assistance in preventing the development of lymphatic pathology which does not become pronounced until after this time (Schacher and Sahyoun, 1967; Rogers and Denham, 1974).

It is clear from the histogram (Fig. 1) that most adult worms are recovered from the lymphatic afferent to the popliteal lymph node after infective larvae have been injected subcutaneously into the hind foot. This has great advantages when studying, for example, pathological changes in the lymphatics after infection or the effects of a potential anthelmintic on adult worms as the post mortem examination can be confined to a single lymphatic. However, approximately 7% of the fourth stage and adult worms are not found in this site and we frequently found a few adult worms in the lymphatics of the tail or in the complicated lymphatics of the pelvic region. In most cats the lymphatics from the foot region and lower leg drain entirely into the popliteal node and in these cats fewer worms were found beyond the popliteal node. Super-imposed on this there are fine lymphatics in the skin, especially of the inner thigh and these occasionally become parasitized by *B. pahangi.*

The pattern of migration shown by Ewert (1971) for *B. malayi* was confirmed for *B. pahangi.* The third stage larvae penetrate the lymphatics near the site of inoculation within a few hours of infection and migrate to the perinodal sinus of the nearest lymph node. Here they stay for about 20 days before returning to the afferent lymphatic draining into the node. They spread themselves along the lymphatic right down to the ankle and cause gross enlargement of the lymphatic.

This migration to the lymph node and then back into the lymphatics accounts for the finding of adult worms in the lymphatics of the tail. Those larvae which manage to pass the popliteal lymph node, either by migrating through the connective tissue before entering the lymphatics, by passing through the node or along a skin lymphatic, then enter the afferent lymphatics of the nodes of the pelvic region. The drainage into the nodes in the pelvic region is most complex and there are numerous cross linkages between the various units. When the larvae migrate away from the lymph nodes they would not necessarily go into the lymphatics which have come from the popliteal node; they could as easily travel into the lymphatics of the tail and even the lymphatics draining the contralateral limb where they would enter the efferent lymphatic draining the popliteal node.

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