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THE RESPONSE OF CAPTIVE RODENTS TO THE MIGRATION OF ANCYLOSTOMA CANINUM LARVAE

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Abstract. The distribution of the larvae of *Ancylostoma caninum* in tissues of captive wild rodents (*Rattus rattus*) and the leucocytic and behavioural responses of these rodents were studied after experimental oral infection. There was a wide distribution of larvae in tissues with a preponderance of the larvae in the skeletal muscles of the anterior part of the body in older infection. The rats responded by an elevation of total leucocytes and eosinophils in blood, alternation of locomotory activity and behavioural dominance that may have a correlation with predation and epidemiology of *A. caninum* in a sylvatic setting.

The migration, distribution and persistence of *A. caninum* larvae in tissues of rodent paratenic hosts were studied under various experimental situations (Matsusaki 1951, Nichols 1956, Soh 1958, Kono and Sawada 1961, Bhopale and Johri 1975, 1978), but the responses of the rodents to the migrating parasites have not been the object of many studies. Furthermore, most of these studies were done on mice and tame laboratory animals and need to be supplemented by work on other rodents and, from the viewpoint of paratenesis, wild rodents which are more relevant in predator-prey relationships. One of such rodents is the black rat (*Rattus rattus*) which was shown to be infected with parasites of veterinary and medical importance in Nigeria (Akinboade et al. 1981). Following our observation (unpublished) that this rodent may allow the migration of *A. caninum*, it is necessary to study the migration and distribution of the larvae of the parasite in the tissues of the black rat and the responses of the rodent to the parasite.

MATERIALS AND METHODS

A. caninum eggs were obtained from the faeces of six puppies of a crossbred bitch faecal samples contained up to 19 000 eggs per gram of faeces. Larval culture and recovery was done by the method of Sellers and Dipeolu (1975).

The rats used were trapped in different areas of Ibadan, Nigeria, and they were selected so that weight difference between chosen individuals was no more than 5 grams. They were kept in cages for at least 6 weeks before the commencement of experiments and the males were kept individually. Commercial rat feed and were provided ad libitum.

Each rat was infected with 1000 *A. caninum* larvae by means of a stomach tube after ether anaesthesia.

In group 1a experiments, the migration, distribution and persistence of larvae in rodent tissues was studied. Six rats were killed with ether 1, 4, 8, 14, 21 and 35 days after infection and the recovery of larvae from rodent tissues was done by the method of Norris (1971). In group 1b experiments, total leucocyte and eosinophil estimations were done by routine methods (Dacie and Lewis 1968) on blood samples obtained from the cut tail-tip of 10 infected and 8 controlled rats every 4 days for 32 days.

In group 2a experiments, the locomotory and exploratory activities of infected rats and their controls were tested in an "open field" which consisted of a perspex box $50 \times 50 \times 40$ cm with a grid of 25 squares 10×10 cm. The number of squares crossed, the number of times they stood

up and the length of time spent grooming were recorded. In group 2b experiments, behavioural dominance was studied between infected and non-infected male rats in neutral cages for a maximum of 20 minutes. The number of attacks and submissions by members of either group were recorded. An attack was defined as an attempt by one male to bite another and submission was indicated by (1) attempted escape (2) unresponsiveness to attack. The ability of rats to maintain behavioural dominance when challenged by intruders was assessed by introducing non-infected male rats into the cages of infected rats and dominance was assessed as described previously. All the infected rats used in behavioural studies were killed at the end of the experiments and tested for the presence of larvae in tissues.

RESULTS

The distribution of *A. caninum* larvae in tissues of *R. rattus* in relation to time after infection is shown in Table 1. There was a shift from a preponderance of larvae in the tissues of the thoracic and abdominal viscera in early infection to the skeletal muscles, especially those of the anterior part of the body in later infection. This shift was most pronounced between the 8th and 14th days of infection, a period which also coincided with that of maximum larvae in tissues. There was a persistently low level of larvae in the brain of the rodents from day 8 onwards. A gradual larval attrition occurred from day 8 such that the number of larvae in tissues on day 35 was 67 % less than on day 1.

Table 1. The distribution of *A. caninum* larvae in *Rattus rattus* tissues

Tissue	Infection period (days)					
	1	4	8	14	21	35
Stomach	212 ± 4.9	34 ± 3.7	6 ± 3.3	—	—	—
Ileum	263 ± 16.5	28 ± 4.1	—	—	—	—
Colon	30 ± 4.5	12 ± 2.6	—	—	—	—
Caecum	10 ± 2.9	6 ± 1.7	—	—	—	—
Liver	134 ± 5.1	22 ± 3.7	—	—	—	—
Spleen	—	4 ± 2.9	—	—	—	—
Kidney	—	—	—	—	—	—
Heart	—	—	—	—	—	—
Lungs	72 ± 4.4	128 ± 4.8	10 ± 3.26	4 ± 1.2	2 ± 1.1	—
Brain	—	—	6 ± 0.8	8 ± 1.6	6 ± 1.8	6 ± 2.5
Skeletal muscles						
Head + neck	8 ± 1.8	300 ± 9.1	332 ± 4.4	238 ± 5.9	188 ± 4.4	92 ± 6.5
Thorax	—	122 ± 3.4	148 ± 5.5	184 ± 3.4	162 ± 4.9	104 ± 6.9
Abdominal	—	—	4 ± 1.6	22 ± 5.5	16 ± 4.2	6 ± 3.7
Fore-limb	—	20 ± 4.1	42 ± 4.1	60 ± 5.2	46 ± 2.4	24 ± 2.9
Hind-limb	—	—	2 ± 1.6	20 ± 4.9	14 ± 4.9	4 ± 2.9
Total	729 ± 15.5	676 ± 12.2	550 ± 8.2	536 ± 8.2	434 ± 3.7	236 ± 9.8

Figs. 1 and 2 represent the total leucocytic and eosinophilic response of the black rat to the migrating larvae respectively. There was an elevation of total leucocyte count which was maximal (42 %) on day 20 and a persistent eosinophilia which reached a peak in 16 days.

There was a significant difference ($P < 0.005$) in the locomotory and exploratory activity of infected and uninfected rats in the "open field test" on days 14 and 35 as expressed by the number of squares crossed, the number of "stand-ups" and the groom-

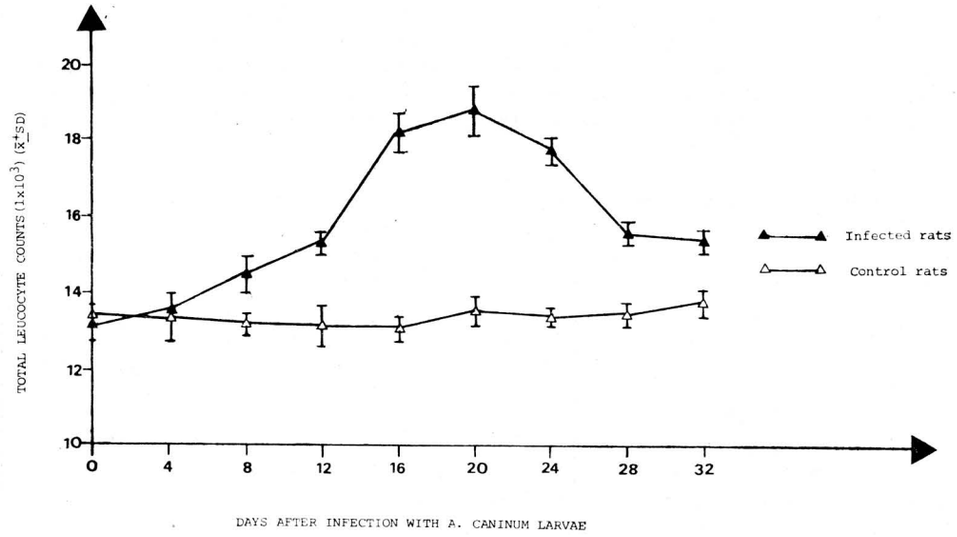

Fig. 1. Total leucocyte counts in captive rats during the migration of *Ancylostoma caninum* larvae.

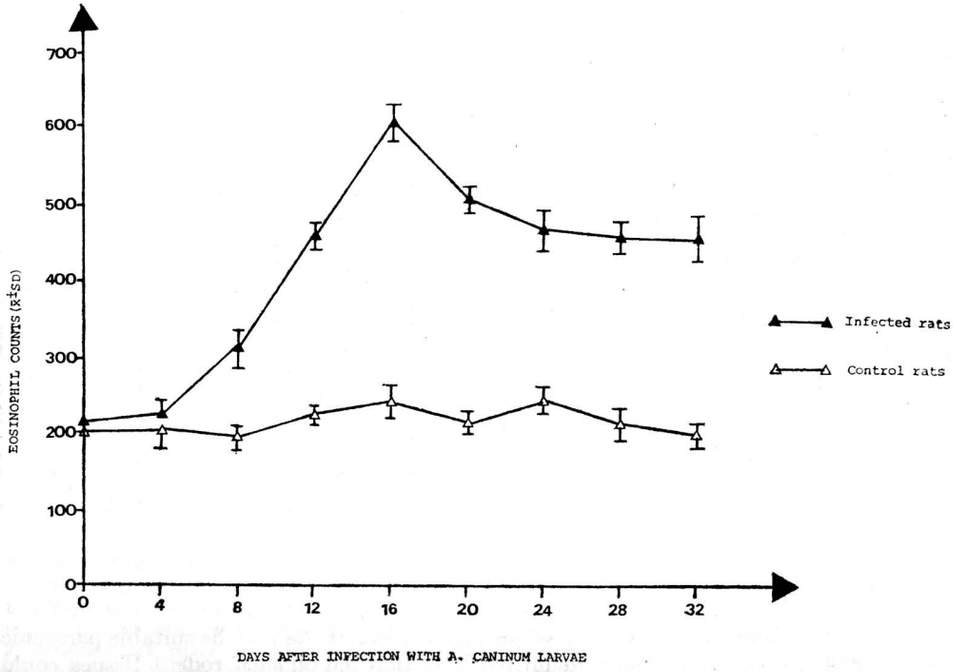

Fig. 2. Eosinophil counts in captive rats during the migration of *Ancylostoma caninum* larvae.

ing time ($P < 0.005$) (Table 2). There were no significant differences on day 4. Table 3 indicates that the presence of *A. caninum* larvae in the tissues of the rodents interfered with the establishment of behavioural dominance in males as judged by the ability of the rats to assert themselves in neutral territory (NT) and to defend a familiar territory (FT) respectively; χ^2 NT = 8.06, DF 1 $P < 0.005$; χ^2 FT = 6.73, DF 1 $P < 0.005$.

Table 2. Effect of *A. caninum* larvae on locomotory and exploratory activities of male *Rattus rattus* in the "Open Field Test"

Day-Post infection		Result		
		Number of squares crossed	Grooming time (sec)	Number of "stand ups"
4	Infected rat	169 ± 10.0	86 ± 2.5	17 ± 2.2
	Control	172 ± 7.4	90 ± 3.3	21 ± 3.0
14	Infected rat	225 ± 5.3	59 ± 3.5	8 ± 2.1
	Control	176 ± 6.4	89 ± 3.2	19 ± 1.8
35	Infected rat	219 ± 3.1	65 ± 2.4	10 ± 1.6
	Control	169 ± 4.2	89 ± 2.9	23 ± 2.3

Table 3. Effect of *A. caninum* larvae on the establishment and maintenance of behavioural dominance in male *Rattus rattus*.

Day post infection	Test	Number of pairs	Number of fights	Submission by control rat	Submission by infected rat
16	Behavioural dominance in mutually unfamiliar territory	15	6	2	13*
21	Behavioural dominance in home cages of infected rats	15	9	3	12**
28	Behavioural dominance in home cages of infected rats	15	7	3	12**
35	Behavioural dominance in mutually unfamiliar territory	15	5	3	12**

* $\chi^2 = 8.06$ DF 1 $P < 0.005$, ** $\chi^2 = 6.73$ DF 1 $P < 0.005$

DISCUSSION

The results obtained in this study suggest that wild rodents may be suitable paratenic hosts for *A. caninum* because a definitive host that fed on such rodent tissues could become infected (Scott 1928). This may be important in the transmission of the nematode among wild carnivores, some of which are suitable definitive hosts for *A. caninum* (Soulsby 1968). The migration and distribution of the larvae in the tissues of *R. rattus* is similar to that in laboratory mice (Soh 1958, Bhopale and Johri 1975) and of *A. braziliense* and *A. tubaeforme* in mice and rats as regards the preferential distribution in the skeletal muscles of the anterior body after an initial preponderance of larvae in the tissue of the gastrointestinal tract, liver and lungs following an oral infection and this may correspond to the direction of migration. The gradual larval attrition observed may be due to the trapping and killing of some larvae in tisseus since such a reaction is normal in an immunocompetent host and the ability of the rodents to respond by eosinophilia and elevation of total leucocyte count implies a manifestation of their ability to mobilise some immune mechanism. Eosinophilia can be related to helminth infections in those nematodes such as *Ascaris* sp., *Strongyloides stercoralis*, *Trichinella* sp. and *Toxocara canis* in which there are tissue-stage larvae (Jaggi and Wishwanathan 1976). The eosinophils are able to migrate into tisseus and destroy helminth stages that are migrating through the tissues (Butterworth 1977). However, despite their attrition, a high number of larvae persisted in the tissues of the rats.

The presence of living larvae in the tissues of the rodents had significant influence on their locomotory activities and behabioural pattern although the mechanisms of this interference are not known. However, the local reactions that they would excite in vital tissues such as the central nervous system and skeletal muscles may cause a physical impairment of function and ill health which may compromise overall activity. The defeat of infected rats by their non-infected counterparts has deep implications. In small mammal communities in which social organization is maintained in part by frequent tests of dominance, the consequences of defeat may include a forced emigration of a subordinate. Such a migrating subordinate may be more exposed to predation (Lidicker 1975). Rau (1983b) observed similar behavioural changes in mice infected with *Trichinella spiralis* and related this finding to the possibility of increased predation of subordinates.

The apparent increase in wandering by infected rats is related to the observation that they spend a shorter time in grooming and standing up. These two latter parameters are indices of familiarisation in new territory. Again the changes of a wandering rat being caught by a predator is higher. For instance, in mice, Metzgar (1967) found that exploring rodents are more exposed to predation in an unfamiliar environment.

Although many workers have reported alterations in activities of parasitized laboratory rodents, *Trichinella spiralis* in rats (Von Brand et al. 1954), *Toxoplasma gondii* in mice (Hutchinson et al. 1980), *Toxocara canis* in mice (Dolinsky et al. 1981) and *T. spiralis* in mice (Rau 1983a, b), the present study adds another model to the group of such relationships.

ОТВЕТ ОТЛОВЛЕННЫХ ГРЫЗУНОВ НА МИГРАЦИЮ ЛИЧИНОК *ANCYLOSTOMA CANINUM*

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Резюме. Изучали распределение личинок *Ancylostoma caninum* в тканях диких грызунов (*Rattus rattus*), поведение этих грызунов и количество лейкоцитов после экспериментального заражения через рот. Личинки широко распространялись в тканях хозяина, большей частью в скелетных мышцах в передней части тела в случае продолжительного заражения. У зараженных крыс наблюдалось повышенное количество лейкоцитов и эозинофилов в крови и изменение движения и поведения, что, вероятно, связано с хищничеством и эпидемиологией *A. caninum* в лесном поселении.

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