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The survival of *Trichuris muris* in wild populations of its natural hosts

J. M. BEHNKE *and* D. WAKELIN

Department of Zoology, Bedford College, London NW 1
and Wellcome Laboratories for Experimental Parasitology,
University of Glasgow, Bearsden, Glasgow G61 1QH

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SUMMARY

The results of experimental infections of *Trichuris muris* in wild field mice (*Apodemus sylvaticus*) and laboratory-bred wild house mice (*Mus musculus*) showed that the parasite elicited an immune response similar to that previously described in strains of laboratory mice. Experiments in laboratory mice showed that the parasite was able to become sexually mature only when small single infections or repeated low-level infections were given. A survey of a population of 43 wild house mice naturally infected with *T. muris* showed that the pattern of small worm burdens in the majority of mice was consistent with a situation of repeated low-level infection, except in the case of six female mice which harboured larger mature worm burdens. It is suggested that in these mice pregnancy and/or lactation may have suppressed the immune response, allowing the accumulation of a worm burden in excess of the threshold for worm expulsion.

INTRODUCTION

An interesting and relatively unstudied aspect of helminth biology is the problem of survival in the wild of species which, when passaged in laboratory strains of their natural host species, provoke strong, protective immune responses. This problem is most acute in parasites such as *Nippostrongylus brasiliensis* and *Trichuris muris* which may, during a primary infection, elicit responses capable of bringing about the expulsion of the worms from the host's intestine. In *N. brasiliensis* adult worms developing from an initial infection are expelled after a short period of egg production (Mulligan, Urquhart, Jennings & Neilson, 1965; Ogilvie, 1965), but in *T. muris*, where the prepatent period is long, the worms are removed before maturation and egg-laying (Wakelin, 1967) and cannot, therefore, contribute to the survival of the species. In nature, however, *T. muris* continues to reproduce itself in populations of wild rodents and there must therefore be some means by which the apparent limitations imposed by the immune response are circumvented. Two such means can be proposed, namely that the host–parasite relationship between *T. muris* and wild rodents differs qualitatively from that which has been described in laboratory mice, and that protective immune responses do not occur to the same extent, or that the level and frequency of infection in the wild is such that protective responses are not elicited.

During the course of a study of nematodes of wild rodents the opportunity arose

of sampling a wild population of house mice (*Mus musculus*) in which *T. muris* was known to occur, and of trapping and infecting specimens of the long-tailed field mouse (*Apodemus sylvaticus*), a recorded host of *T. muris*, in which the parasite did not occur. In this way it was possible to add information to that available from infections of laboratory mice and laboratory-bred house mice in order to test the hypotheses outlined above.

MATERIAL AND METHODS

Laboratory mice

Male mice of the Carworth-Europe CFLP (specific pathogen free) strain were used and were infected when approximately 6 weeks of age unless stated otherwise. The mice were maintained on a sawdust litter and had access to pellet food and water *ad libitum*.

Field mice

The *Apodemus* were trapped live, using baited Longworth traps, in the grounds of Trent Park College, Middlesex. Traps were set in the evening and examined the following morning. Mice were removed to the laboratory, sexed, weighed and maintained as described above. To facilitate handling and infection the mice were lightly anaesthetized with ether. Only mature mice were used for the experimental infections and these were given piperazine citrate orally, at a dose rate of 500 mg/kg body weight, in order to remove existing burdens of the nematode *Syphacia stroma*. *Nematospiroides dubius*, *Corrigia vitta* and two species of cestodes were also present in individuals from the population, but *T. muris* was never recorded. The mice were infected 3 days after injection with piperazine citrate.

Laboratory-bred house mice

A number of male and female mice from a colony maintained in the Zoology Department of the University of Glasgow were kindly made available by Mr R. Stoddart. The colony was established from trapped wild mice and has been bred in the laboratory for more than 20 generations. The mice were lightly infected with *Aspiculuris tetraptera* and *Syphacia obvelata*, but no attempt was made to remove these before infection with *T. muris*. Anaesthesia with ether was used in order to facilitate handling.

House mice

These were caught in domestic premises using baited break-back and Longworth traps. The mice were sexed, weighed and the intestines removed and carefully examined for helminths under a binocular dissecting microscope. Infection with parasites other than *T. muris* was rare in the population; *Syphacia obvelata* and the strobilocercus larva of *Hydatigera taeniaeformis* were each recorded on one occasion.

Methods used in the maintenance of *T. muris* and for the infection and examination of mice have been described in a previous paper (Wakelin, 1967). Two cultures of the parasite were used in the infections, one recently isolated from naturally infected house mice, the other a long established laboratory strain, passaged in laboratory mice for over 20 years.

Table 1. *The development of* T. muris *in mice given single or repeated (trickle) infections of eggs*

Group	No. of eggs	Nos. of worms recovered: At 2 weeks Mean	At 2 weeks S.D.	At 6 weeks Mean	At 6 weeks S.D.	Analysis of worm recoveries at 6 weeks: No. of mice with worm burdens > 2-week mean for dose	No. of mice with adult worms and mean worm burden		No. of mice with larval worms and mean worm burden	
1	50 × 1	22·3	4·29	1·4	1·39	0/9	1/9	1·0	6/9	2·0
2	25 × 1	12·7	1·41	3·7	3·77	0/9	5/9	5·0	4/9	2·0
3	10 × 1	3·7	1·63	4·0	1·00	6/9	7/9	3·4	5/9	2·2
4	10 × 5	Not done		3·3	4·08	0/9	6/9	2·3	5/9	3·2
5	5 × 5	Not done		6·1	3·30	0/9	6/9	2·6	8/9	4·6
6	2 × 25	Not done		10·8	10·36	3/8	5/9	6·6	6/9	10·7
7	2 × 12	Not done		3·7	4·62	1/9	3/9	3·3	5/9	4·6

RESULTS

The establishment of single and repeated (trickle) infections in laboratory mice

It has been shown previously (Wakelin, 1973) that the worms which develop from a single low-level infection (< 15 eggs) are not removed from the host by a primary self-cure response, but persist and become sexually mature. When trickle infections of 10 or 5 eggs were given daily over a period of 2 weeks or so a primary response was elicited and the worms removed. A daily intake of 10 or even 5 embryonated eggs may be an unrealistic model of the natural situation and it was therefore decided to amplify the trickle infection experiment. Seven groups, each of 12 CFLP mice were infected with the laboratory culture of *T. muris* as follows:

Groups 1–3. A single infection of 50, 25 or 10 eggs.

Groups 4 and 5. Five infections of 10 or 5 eggs given at weekly intervals.

Groups 6 and 7. Daily infections with 2 eggs given for 25 or 12 days.

Thus three infection levels, namely 50, 25 and 10 eggs were tested, the first two levels being achieved both as single and as divided doses. Three mice from each of groups 1, 2 and 3 were killed 2 weeks after infection and all remaining mice were killed 4 weeks later. The results are given in Table 1.

The results confirm previous studies and show that a primary response was elicited when more than 10 eggs were given as a single infection. In groups 1–3 approximately 50% of the infection had become established by the second week, but in both groups 1 and 2 the mean worm recovery at 6 weeks after infection was considerably reduced. In group 1 the worms remaining after the immune response had taken place were small and stunted, the characteristic situation which occurs when self-cure is induced by much larger infections. The establishment and fate of worms from the trickle infection groups is less straightforward to interpret. Worms established from the first two infections in groups 4 and 5 and from the first 7 to 10 infections in groups 6 and 7 would have had sufficient time in which to become mature and thus, in the absence of an immune response, each mouse should have

harboured at 6 weeks a small number of adult worms (approximately equal to the 2-week mean of group 2 [25 eggs] in the cases of groups 4, 6 and 7, and of group 3 [10 eggs] in the case of group 5) and a larger number of immature worms, the total burden approximating to the 2-week mean of group 1 (50 eggs) in the cases of groups 4 and 5, and of group 2 (25 eggs) in the cases of groups 5 and 7. By this criterion none of the trickle regimes was successful in avoiding the stimulation of an immune response. The high mean burdens, both of adult and larval worms, in group 6 were the result of high counts from 3 mice, which contributed a total of 24 adults and 48 larval worms, and thus the mean values are not representative. This is borne out by the low values in group 7, although this group too contained two mice with relatively high burdens. The most successful regime in terms of adult and larval burdens established and of consistency of infection in the mice was that of group 4, namely five infections each of 5 eggs, although none of the burdens of individual mice was higher than the 2-week mean for group 2. It therefore seems reasonable to conclude that a threshold value for the stimulation of an immune response is reached even when small numbers of eggs are administered at intervals and that this threshold is reached when some 10 to 20 worms are established. However, there is some indication that the immune response stimulated by trickle infections is qualitatively different from that stimulated by single large infections, in that the first worms to become established from a trickle infection may mature even though worms established later are eliminated. For example in group 3, seven of the nine mice had mature worms, but only four of these had larval worms. The mean values for mature and larval worms are similar, although if there had been no response there should have been many more larvae. In group 4, six of the nine mice had mature worms, with a mean value indicating that almost all of the worms established by the first two infections had survived. In contrast, however, the mean number of larval worms was lower than the expected value. This differential survival may be dependent upon the frequency of infection and the total number of eggs given, as trickle infection regimes used previously have brought about a near-complete expulsion of the infection (Wakelin, 1973).

Comparison of the development of low- and medium-level infections in Apodemus sylvaticus *and CFLP laboratory mice*

Apodemus sylvaticus has been reported as a natural host of *T. muris* by several authors (Dujardin, 1845; Bernard, 1964; Wertheim, 1971). The population from which the experimental field mice were taken was adjudged to be *Trichuris*-free on the basis of examination of 26 animals. A total of 22 *Apodemus* and 19 CFLP mice were infected with eggs taken from the recently isolated culture of *T. muris* and two levels of infection were used: namely (*a*) 300 eggs, at which level a primary self-cure response is elicited in CFLP mice and (*b*) 5 eggs at which level no response is elicited. Non-pregnant female mice were used for the higher level infection and male for the lower; there is no sex difference in response to *T. muris* in laboratory mice. Five *Apodemus* and three CFLP mice infected with 300 eggs were killed 2 weeks after infection, in order to ascertain the size of the established worm

Table 2. *Comparison of the development of* T. muris *in* Apodemus sylvaticus *and CFLP laboratory mice given infections of* 5 *or* 300 *eggs*

Level of infection	Host	Number of worms recovered after infection: After 2 weeks Mean	After 2 weeks S.D.	After 5 weeks Mean	After 5 weeks S.D.	% adult worms at 5 weeks
5 eggs	CFLP	Not done		1·8	1·5	72
	Apodemus	Not done		1·9	1·2	74
300 eggs	CFLP	193·0	35·7	11·7	10·8	0
	Apodemus	167·4	87·4	3·7	5·6	0

population. The remaining mice from both infection levels were killed 3 weeks later. The results are shown in Table 2.

The infection of 300 eggs became established equally well in *Apodemus* and CFLP mice, but in both species the majority of worms were lost at some point after the second week. None of the surviving worms had reached sexual maturity or even the juvenile stage of development. In contrast, the majority of worms recovered from the *Apodemus* and CFLP mice infected with five eggs were sexually mature and eggs were present in faecal samples taken on day 35, the normal time of patency. Only one animal (an *Apodemus*) in the groups infected at this level was without worms. The behaviour of the two host species with respect to low- and medium-level infections of *T. muris* therefore seems to be comparable.

Development of Trichuris muris *in laboratory-bred wild house-mice*

Five male and 14 female mice were available for infection. All were given an infection of 400 eggs from the laboratory strain of *T. muris*. Four female mice were killed 14 days after infection, when the mean worm burden was 141·0; the remaining mice were killed 3 weeks later. None of the male mice had worms present and only one of the female mice was infected, although this mouse had a fully mature burden of worms. It appears, therefore, that an immune response to the parasite had developed after the second week of infection and had eliminated the worm population in the majority of the mice, a situation which is directly comparable to that in laboratory mice.

Survey of Trichuris muris *in a population of wild house mice*

T. muris has been recorded in populations of house mice on very few occasions (Fahmy, 1954; Roman, 1951). The discovery of a source of infected mice in domestic premises provided an opportunity to assess the incidence and intensity of infection under natural conditions in order to establish whether the distribution of the parasite would be as predicted from the results of the above experiments, namely:

(*a*) That mice would harbour small numbers of mature and/or larval worms, corresponding to a situation in which infection had been acquired by ingestion of a small total (subthreshold or threshold) number of eggs.

Table 3. *The occurrence of* T. muris *in a population of wild house mice*

		Distribution of infections						
		Uninfected	Infected < 20 worms			Infected > 20 worms		
	No. of mice examined	No. of mice	No. of mice	Mean no. worms	% adult worms	No. of mice	Mean no. worms	% adult worms
Mature ♂	8	1	7	8·6	43·3	0	—	—
Juvenile ♂	10	2	8	3·4	0·0	0	—	—
Mature ♀ (non-pregnant)	9	0	7	5·0	57·1	2	81·5	61·4
Mature ♀ (pregnant)	8	0	4	4·6	69·5	4	61·3	59·6
Juvenile ♀	8	2	6	1·9	0·0	0	—	—
Total mice	43	5	32	—	—	6	—	—

(*b*) That mice would harbour a few immature worms, corresponding to a situation in which sufficiently large numbers of eggs had been ingested to elicit a normal self-cure response and subsequent immunity.

The results of the survey are given in Table 3.

DISCUSSION

Experimental studies in laboratory mice have shown that *T. muris* is a strongly immunogenic parasite, capable of eliciting a self-cure response in its host (Wakelin, 1967). The response takes place during the second or third week of a primary infection (depending on the strain of mouse) and thus, as *T. muris* has a prepatent period of approximately 5 weeks, prevents the worm from reaching sexual maturity. Immunity once acquired is long-lasting and protects against subsequent infection for at least 3 months. In view of these facts the propagation of *T. muris* in wild populations of its host species becomes problematical. There are, however, a number of possible explanations for the survival of *T. muris*, which may be summarized as follows:

(*a*) Under natural conditions animals ingest relatively few embryonated eggs and do not acquire a worm population large enough to elicit an immune response.

(*b*) The laboratory situation is artificial and the response of wild hosts to wild strains of the parasite differs qualitatively from that of laboratory mice to laboratory-adapted strains of the parasite.

(*c*) Wild host species contain individual animals which are unresponsive to *T. muris* and in which the parasite can mature and reproduce.

The first experiment described in this paper demonstrates that there is indeed a threshold worm population size, below which a primary self-cure response does not occur, and it is suggested that this threshold lies between 10 and 20 worms. Worms established from a subthreshold infection mature and the infection becomes patent. Trickle infections, in which small numbers of eggs are given repeatedly, do not circumvent the development of a self-cure response, as is apparently the case in

Nippostrongylus brasiliensis (Jenkins & Phillipson, 1971), but elicit a response when the population threshold is reached. However, in this situation not all the worms are eliminated and a small number become sexually mature. This situation resembles that in *N. brasiliensis* when trickle infections of 50 larvae per day are given; a self-cure occurs, but some worms remain and resume egg production.

The development of experimental infections above and below the threshold level in wild *Apodemus sylvaticus* and laboratory-bred house mice seems to be directly comparable to the situation in laboratory mice. The infections in *Apodemus* were established using a culture of *T. muris* isolated from infected house-mice and passaged for only a short time in laboratory mice, so that there could be no question of using an atypical, laboratory-adapted strain of the parasite. The *Apodemus* responded uniformly to infection at both levels used; none of the seven mice infected with 300 eggs and killed after 5 weeks was found to be unresponsive, i.e. had allowed the worm population to persist and mature, as is the case with individual animals in certain strains of laboratory mice (Wakelin, 1970), although one unresponsive animal was revealed in the laboratory-bred house mice. The experiments suggest, therefore, that *T. muris* is unlikely to reach sexual maturity in wild populations unless infection rates are low or unless some other factor intervenes to suppress the animal's normal immune responses.

The results of the survey are striking, in that the majority (88%) of the wild house mice were infected with *T. muris* and that, in mature mice, the percentage of adult worms lay between 43 and 70%. Mature worms would not be expected in juvenile mice, less than 6 weeks of age, as it appears that mice cannot be infected before the age of 2 weeks (Wakelin, 1973). Most of the infected mice (84%) had burdens of less than 20 worms, a situation which, taken together with the proportion of mature worms, could be explained as a result of exposure to small numbers of embryonated eggs, leading to the acquisition of subthreshold infections, or as a result of exposure to somewhat larger numbers of eggs leading to the type of immune response that was demonstrated in experimental trickle infections. This view is supported by the fact that the juvenile mice had low worm burdens, as it is less likely that there would have been sufficient time for these mice to acquire burdens large enough to elicit a self-cure response and thus their burdens should reflect the level of infection to which the population was exposed. The existence of burdens greater than 20 worms in six mature female mice [non-pregnant 43 and 120; pregnant 29, 31, 54 and 131] could be consistent with this explanation if it is assumed that the females were either genetically unresponsive to the parasite or were physiologically unresponsive, as a result of pregnancy and lactation, and had therefore not limited the size of the population of parasites with which they were infected. The fact that only female mice were found to harbour large infections would suggest that physiological unresponsiveness was the more likely explanation. In wild mice adult females are normally pregnant and/or lactating [R. Stoddart, personal communication] and there is good evidence that these states suppress the immune responses of animals to helminth parasites (reviewed by Dineen & Kelly, 1972). In rats infected with *Nippostrongylus brasiliensis* lactation has the effect of delaying worm expulsion in both primary and challenge infections (Connan, 1970,

1972) and preliminary experiments in laboratory mice have shown that self-cure is suppressed when mice are infected with *T. muris* during lactation, although infections given during pregnancy appear to stimulate a normal immune response (G. R. Selby & D. Wakelin, unpublished). It is therefore obvious that a reproducing population of wild host animals may well contain a number of females carrying mature worm burdens adequate to provide sufficient infective eggs for the propagation of *T. muris*, and in this case the numbers of eggs produced by mature worms from mice exposed to subthreshold infections would be of minor importance. However, as the survey shows, not all mature females are heavily infected with the parasite and the eggs from other infected members of the population may well be the long-term means of survival of *T. muris*.

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