Host specificity of and cross-immunity between two strains of *Heligmosomoides polygyrus*

R. J. QUINNELL[1], J. M. BEHNKE[2]* *and* A. E. KEYMER[1]

[1] *Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS*
[2] *Department of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD*

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SUMMARY

The infectivity of wild and laboratory strains of *Heligmosomoides polygyrus* (*Nematospiroides dubius*) in laboratory mice and in three species of wild British rodent was compared. Wild strains, of the subspecies *H. p. polygyrus*, were isolated from wild caught *Apodemus sylvaticus*. Only very low-level infections of the wild strains became established in laboratory mice. Similar worm burdens of the laboratory strain became established in laboratory mice and *A. sylvaticus*, although infections in *A. sylvaticus* were more short lived. Cortisone treatment of hosts increased the establishment and survival of the heterologous worm strain to that of the homologous strain. In contrast, neither strain of parasite established in *Clethrionomys glareolus* or *Microtus agrestis*, and cortisone treatment of *C. glareolus* did not increase establishment. Infection of laboratory mice with the wild-strain parasite induced significant immunity to a challenge infection with the laboratory strain.

Key words: *Heligmosomoides polygyrus*, *Nematospiroides dubius*, *Apodemus sylvaticus*, *Clethrionomys glareolus*, *Microtus agrestis*, infection, infectivity, host specificity, Nematoda, immunity.

INTRODUCTION

Heligmosomoides polygyrus (Dujardin, 1845) (= *Nematospiroides dubius* Baylis, 1926) is a common parasite of wild rodents throughout the Holarctic region. The taxonomy of the genus *Heligmosomoides* has been confused, and *H. polygyrus* has been variously recorded as *H. polygyrus*, *H. skrjabini*, *Heligmosomum polygyrum*, *H. dubium*, *H. costellatum*, *H. azerbaidjani*, *H. kratochvili*, *Sincosta aberrans* and *Nematospiroides dubius*, which are all now considered synonyms (Tenora, 1966; Durette-Desset, 1968*a b*). Four subspecies of *H. polygyrus* have been described: *H. p. polygyrus* and *H. p. corsicus* from Europe, the latter restricted to Corsica, and *H. p. bakeri* and *H. p. americanus* in North America (Durette-Desset, Kinsella & Forrester, 1972). The subspecies can be distinguished morphologically, and also differ in host range. *H. p. polygyrus* usually occurs in *Apodemus*, and less commonly *Mus*; it is also an occasional parasite of cricetids (see Discussion section). The other three subspecies are thought to derive from *H. p. polygyrus* infections in *Mus musculus*; *H. p. corsicus* is restricted to *Mus musculus*, *H. p. bakeri* is a common parasite of *Mus*, but also occasionally infects *Reithrodontomys* and *Peromyscus*, while *H. p. americanus* is found only in *Phenacomys* (Kinsella, 1967; Durette-Desset *et al.* 1972).

* Reprint requests to Dr J. M. Behnke.

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The host range of *H. p. polygyrus* in Europe comprises 9 species of rodents: *Mus musculus*, 4 *Apodemus* spp., *Rattus norvegicus*, *Microtus agrestis* and 2 *Clethrionomys* spp. (Hannah, 1983). In Britain it is a common parasite of *Apodemus sylvaticus*, occurring at prevalences of up to 90% (Elton *et al.* 1931; Lewis, 1968*a*; Lewis & Twigg, 1972). It has also been reported from *A. flavicollis*, *M. musculus*, *C. glareolus* and *M. agrestis* (Lewis & Twigg, 1972; Lewis, 1987). The factors which could be important in determining this host range are numerous, and include behavioural and immunological characteristics of the hosts. This paper reports an investigation of some of the factors involved. Laboratory infections with three isolates of the wild *H. p. polygyrus* and a laboratory strain were carried out in a range of host mammal species. The laboratory strain was originally isolated from *Peromyscus maniculatus* (Ehrenford, 1954) and corresponds to *H. p. bakeri* (Durette-Desset *et al.* 1972; Behnke & Wakelin, 1977). Finally, cross-immunity between the two strains was examined in laboratory mice.

MATERIALS AND METHODS

Host

Inbred laboratory mice were obtained from Harlan Olac Ltd, Bicester, Oxon. *Apodemus sylvaticus* were wild-caught in Woodchester-Stroud, Glos. (Stroud

strain), Cotgrave Wood, near Nottingham (Nottingham strain) or Wytham Woods, near Oxford (Oxford strain) and treated with ivermectin (10 mg/kg, Stroud and Nottingham strains) or pyrantel embonate (100 mg/kg, Oxford strain) several weeks before use. *Clethrionomys glareolus* and *Microtus agrestis* were obtained from breeding populations in the Department of Zoology, Oxford. This was set up in 1958 with locally wild-caught animals, and has since been maintained as described by Baker & Clarke (1987). Faecal smears of 20 animals of each species showed there to be no *H. polygyrus* present in the colony. *C. glareolus* used in Nottingham were wild-caught, and treated before use with pyrantel embonate as above. Limited numbers of laboratory bred voles were available for the project and the use of wild caught animals was restricted as far as was possible. Although every precaution was taken to ensure that groups of animals were comparable, the restriction on the availability of wild caught rodents and laboratory bred voles, and the difficulty of determining their age accurately, made it impossible for these animals to be sex and age matched with laboratory house mice. Despite these limitations, the experiments were carried out under uniform laboratory conditions and the important trends were repeated and confirmed in years when wild caught rodents were more readily available.

Parasite

The laboratory strain of *H. polygyrus* was obtained in 1983 (Oxford) and 1975 (Nottingham) from the Wellcome Research Laboratories, Beckenham, Kent, where it has been maintained since 1956. It has since been routinely maintained in MF1 and CD1 mice (Oxford) or CFLP mice (Nottingham), as described by Keymer & Hiorns (1986) and Behnke & Wakelin (1977). Wild strains of *H. polygyrus* were isolated from wild-caught *A. sylvaticus*. The Oxford strain was derived from 4 infected mice trapped in October 1986, and has since been maintained in treated, wild-caught *A. sylvaticus* under the same conditions as the laboratory strain. The Stroud and Nottingham strains were isolated in April 1987 and 1977–1981 respectively; larvae developing from the faeces of wild mice were used without passage.

Experimental procedures

Methods used to infect mice and to recover and count worms were as described by Jenkins & Behnke (1977). Encysted larval worms were counted according to the method of Slater & Keymer (1986). Faecal egg counts were carried out using a modified McMaster flotation technique (Dunn & Keymer, 1986). In Exps 5–7 cortisone acetate (Cortistab, Boots Ltd, Nottingham) was given by subcutaneous injection every second day. The first two injections were at 2·5 mg/mouse and the remainder at 1·25 mg/mouse. All the mice in these experiments were given 3 g/l oxytetracycline hydrochloride (Terramycin, Pfizer Ltd, Sandwich, Kent) in their drinking water.

EXPERIMENTAL DESIGN AND RESULTS

Survival and fecundity of wild and laboratory strains of H. polygyrus *in inbred laboratory mice (C57BL/10, CFLP and NIH) and* Apodemus sylvaticus

These experiments were carried out independently using the Stroud, Nottingham and Oxford strains of *H. polygyrus* in Nottingham (Stroud and Nottingham) and Oxford. In Nottingham, groups of 6 male, 4-month-old C57BL/10 mice (Stroud strain) or groups of 4 CFLP mice (Nottingham strain) were used, and in Oxford groups of 6 female, 2-month-old C57BL/10 and NIH mice were used. Groups of 3 to 6 *A. sylvaticus* of mixed sex and unknown age were used in each experiment.

The results of these experiments are shown in Table 1. Very few (< 10%) of the wild strain larvae developed in laboratory mice of the 3 strains, and worm burdens were significantly lower than those in *A. sylvaticus* or those of laboratory strain larvae in laboratory mice at all time points (Mann-Whitney U-test, $P < 0{\cdot}05$). In Exp. 2 there were no significant differences between worm burdens in NIH and C57BL/10 mice infected with either the wild or laboratory strains of parasite (Mann-Whitney U-test, $P > 0{\cdot}05$). There was no significant difference in the number of laboratory strain worms in C57BL/10 mice and *A. sylvaticus* at day 14 in Exp. 1, but there were significantly fewer laboratory strain worms in *A. sylvaticus* than laboratory mice at day 37 in Exp. 1 and at day 14 in Exp. 3 (Mann-Whitney U-test, $P < 0{\cdot}01$ and $P < 0{\cdot}05$ respectively).

There was no significant loss of worms from NIH or C57 BL/10 mice during the 37 (Exp. 1) or 30 day (Exp. 2) period of observation when the laboratory strain was administered. However, there was a significant reduction in worm burden between days 14 and 37 post-infection when laboratory larvae were administered to *A. sylvaticus* (Mann-Whitney U-test, $P < 0{\cdot}05$). Despite the suggestion of a loss of wild-strain worms from *A. sylvaticus* (Exp. 1), this loss was not significant, and both Stroud and Oxford strains established stable, albeit low intensity, infections in C57BL/10 mice. In contrast, NIH mice rejected the Oxford strain by day 15 and CFLP mice given the Nottingham strain had no worms on day 14.

The fecundity of worms of the Oxford and laboratory strains in Exp. 2 is shown in Table 2. At day 30 there were no significant differences in egg production per female worm with either parasite strain or host species (ANOVA, $P > 0{\cdot}05$).

Table 1. Survival of wild and laboratory strains of *Heligmosomoides polygyrus* in laboratory mice and wild-caught *Apodemus sylvaticus*

Exp. 1: Stroud *H. polygyrus*

Mice	Larvae	Dose (mean ± S.E.M.)	No. of *H. polygyrus* recovered (mean ± S.E.M.) Day 14	Day 37
C57BL/10	Stroud	92 ± 3	2·0 ± 0·7	1·2 ± 0·3
C57BL/10	Laboratory	93 ± 6	74·8 ± 6·6	66·7 ± 6·0
A. sylvaticus	Stroud	92 ± 3	50·8 ± 14·2 (1♂, 3♀)*	22·4 ± 10·5 (3♂, 2♀)
A. sylvaticus	Laboratory	93 ± 6	60·0 ± 4·9 (2♂, 1♀)	14·7 ± 7·0 (4♂, 2♀)

* Number and sex of mice in group.

Exp. 2: Oxford *H. polygyrus*

Mice	Larvae	Dose (mean ± S.E.M.)	No. of *H. polygyrus* recovered (mean ± S.E.M.) Day 9	Day 14	Day 12	Day 15	Day 30
NIH	Oxford	51 ± 4	5·2 ± 1·2	1·3 ± 0·6	0·5 ± 0·5	0	0
C57BL/10	Oxford	54 ± 4	2·5 ± 1·9	1·3 ± 1·3	3·5 ± 0·5	0·2 ± 0·2	4·7 ± 2·6
NIH	Laboratory	51 ± 4	48·8 ± 4·6	—	—	44·0 ± 2·5	43·0 ± 4·2
C57BL/10	Laboratory	50 ± 4	43·5 ± 3·4	—	—	45·3 ± 6·0	40·8 ± 2·6
A. sylvaticus	Oxford	40 ± 4	—	—	—	—	30·0 ± 7·5*

* 3 mice in group.

Exp. 3: Nottingham *H. polygyrus*

Mice	Larvae	Dose (mean ± S.E.M.)	No. of *H. polygyrus* recovered on day 14 (mean ± S.E.M.)
CFLP	Nottingham	60 ± 4	0
CFLP	Laboratory	60 ± 4	39·0 ± 3·3
A. sylvaticus	Nottingham	60 ± 4	18·5 ± 2·2
A. sylvaticus	Laboratory	60 ± 4	9·0 ± 2·9

Table 2. Fecundity of wild and laboratory strains of *Heligmosomoides polygyrus* in inbred laboratory mice and wild-caught *Apodemus sylvaticus* (Exp. 2)

(*a*) Total egg production/day

Mice	Larvae	Faecal egg output/day (mean ± S.E.M.) Day 9	Day 12	Day 15	Day 30
NIH	Oxford	—	—	0	0
C57BL/10	Oxford	0	153 ± 153	0	5750 ± 3984
NIH	Laboratory	—	—	41415 ± 2475	29658 ± 4415
C57BL/10	Laboratory	—	—	15400 ± 4166	27708 ± 2007
A. sylvaticus	Oxford	—	—	—	6089 ± 2158

(*b*) Egg production/day/female worm

Mice	Larvae	Faecal egg output/day/female worm (mean ± S.E.M.) Day 9	Day 12	Day 15	Day 30
NIH	Oxford	—	—	—	—
C57BL/10	Oxford	0	66	0	1425 ± 493
NIH	Laboratory	—	—	1794 ± 139	1341 ± 237
C57BL/10	Laboratory	—	—	791 ± 255	1413 ± 98
A. sylvaticus	Oxford	—	—	—	477 ± 234

Table 3. Survival of wild and laboratory strains of *Heligmosomoides polygyrus* in cortisone-treated laboratory mice and *Apodemus sylvaticus*

Exp. 4: Stroud *H. polygyrus*

Mice	*n*	Larvae	Treatment	Dose (mean ± S.E.M.)	No. of *H. polygyrus* recovered on day 14 (mean ± S.E.M.)
C57BL/10	6	Stroud	None	92 ± 3	2·0 ± 0·7
C57BL/10	6	Stroud	Cortisone acetate	92 ± 3	43·7 ± 9·7

Exp. 5: Nottingham *H. polygyrus*

Mice	*n*	Larvae	Treatment	Dose (mean ± S.E.M.)	No. of *H. polygyrus* recovered on day 14 (mean ± S.E.M.)
CFLP	4	Nottingham	None	60 ± 4	0
CFLP	5	Nottingham	Cortisone acetate	60 ± 4	30·0 ± 2·1

Exp. 6: Laboratory *H. polygyrus*

Mice	*n*	Larvae	Treatment	Dose (mean ± S.E.M.)	No. of *H. polygyrus* recovered on day 30 (mean ± S.E.M.)
A. sylvaticus	3	Laboratory	None	200 ± 13	0·5 ± 0·5
A. sylvaticus	3	Laboratory	Cortisone acetate	200 ± 13	118·0 ± 60·5
CFLP	3	Laboratory	None	200 ± 13	161·5 ± 3·5

Exp. 7: Oxford *H. polygyrus*

Mice	Larvae	Treatment	Dose (mean ± S.E.M.)	No. of *H. polygyrus* recovered (mean ± S.E.M.)				
				Day 6	Day 12	Day 15	Day 20	Day 28
NIH	Oxford	None	102 ± 5	1·4 ± 0·9	0·4 ± 0·2	—	—	0·2 ± 0·2
NIH	Oxford	Cortisone (d9-d28)	102 ± 5	—	0·2 ± 0·2	0 ± 0	0·2 ± 0·2	0·2 ± 0·5
NIH	Oxford	Cortisone (d0-d28)	102 ± 5	47·8 ± 5·6	62·6 ± 5·3	88·8 ± 5·2	74·8 ± 7·9	42·4 ± 10·5
NIH	Oxford	Cortisone (d0-d15)	102 ± 5	—	—	—	75·0 ± 9·2	19·8 ± 18·4
NIH	Laboratory	None	106 ± 4	72·6 ± 3·0	—	—	—	99·0 ± 8·7
NIH	Laboratory	Cortisone (d0-d28)	111 ± 3	74·2 ± 2·9	—	—	—	78·0 ± 4·3
A. sylvaticus	Oxford	None	102 ± 5	49·5 ± 16·7	—	—	—	15·0 ± 15·0

The survival of wild and laboratory strains of H. polygyrus *in mice treated with cortisone acetate*

In order to distinguish between innate insusceptibility and immunological resistance to infection, 3 pilot experiments were carried out using the Nottingham or Stroud strains, and the results confirmed and amplified in a larger experiment completed in Oxford. The results of all 4 experiments are presented in Table 3.

As in the previous experiments, untreated laboratory mice given wild strains of larvae from all 3 localities had very few worms at autopsy. Cortisone treatment (from day 0) significantly increased the survival of wild larval strains in laboratory mice in Exps 4, 5 and 7 (Mann-Whitney U-test, $P < 0{\cdot}01$), and there was no significant difference in the survival of wild strain worms to day 6 between cortisone treated laboratory mice and untreated wood mice in Exp. 7 (Mann-Whitney U-test, $P > 0{\cdot}05$). Likewise, cortisone treatment enhanced the survival of the laboratory strain in *A. sylvaticus* in Exp. 6 (Mann-Whitney U-test, $P < 0{\cdot}05$). The survival of laboratory strains in laboratory mice was not affected by cortisone treatment.

In Exp. 7 there was no worm loss from cortisone-treated NIH mice by day 28, although there was a significant loss of worms from *A. sylvaticus* (Mann-Whitney U-test, $P < 0{\cdot}05$). However, when cortisone treatment was stopped on day 15, there was a significant loss of wild-strain worms between days 20 and 28 (Mann-Whitney U-test, $P < 0{\cdot}05$). When the start of cortisone treatment was delayed until day 9 post-infection, the treatment failed to increase worm survival, and there were no significant differences in the wild-strain worm burden of treated and untreated laboratory mice at day 28.

Cross-immunity between wild and laboratory strains of H. polygyrus

This experiment (Table 4. Exp. 8) was carried out to see if a primary infection with a wild (Stroud) strain of *H. polygyrus* could immunize against challenge

Table 4. Effect of anthelminthic-abbreviated primary infections of wild and laboratory strain *Heligmosomoides polygyrus* on the development of a challenge infection with the laboratory strain (Exp. 8)

Group	*n*	Primary infection	Ivermectin day 6	Pyrantel day 27–28	Challenge day 35	Autopsy day	Mean worm recovery ±S.E.M.
A	6	None	Yes	Yes	Yes	70	81·5±10·3
B	6	Stroud	Yes	Yes	Yes	70	15·7±14·5
C	6	Laboratory	Yes	Yes	Yes	70	0·3±0·2
D	6	Stroud	No	Yes	Yes	70	30·5±14·7
E	6	Laboratory	No	Yes	Yes	70	58·8±12·7
F	4	Laboratory	No	No	No	14	108·0±6·0
G	4	Stroud	No	No	No	14	2·0±0·7
H	6	Stroud+ cortisone	No	No	No	14	65·5±6·3

Table 5. Survival of *Heligmosomoides polygyrus* in experimental infections of *Clethrionomys glareolus* and *Microtus agrestis*

Mice	*n*	Larvae	Dose	Mean ±S.E.M. *H. polygyrus* recovered Day 14	Day 42–45
Exp. 9:					
CD1	8	Laboratory	62±3	47±2	—
M. agrestis	4	Laboratory	65±5	0	—
M. agrestis	6	Oxford	75±1	0	—
Exp. 10:					
C. glareolus	6	Laboratory	74±5	0	—
C. glareolus	2	Oxford	53±3	0	—
Exp. 11:					
CFLP	6	Laboratory	100±7	—	81±9
A. sylvaticus	5	Laboratory	100±7	—	0
C. glareolus	5	Laboratory	100±7	—	0
Exp. 12:					
CFLP	3	Laboratory	200±11	133±18	160±10
C. glareolus (cortisone-treated)	4—5	Laboratory	200±11	0	0

with the laboratory strain. Groups of female, 2-month-old NIH mice were infected with wild or laboratory strain larvae, and these infections terminated by anthelminthic treatment with ivermectin (10 mg/kg) 6 days post-infection, or by pyrantel embonate (100 mg/kg) 27–28 days post-infection. Groups which received ivermectin on day 10 were also treated with pyrantel on days 27–28, since this latter drug was administered close to the day of challenge and it was necessary to ensure that any residual drug activity (none was expected given the time interval) affected all groups equally. Groups F, G and H were control groups monitoring the infectivity of the inoculum used at immunization. Worm recoveries from these groups show that the laboratory strain established well (Group F) and that the wild (Stroud) strain did not survive until day 14 (Group G), the latter reinforcing earlier results. However, mice infected with the Stroud strain but treated with cortisone, had substantial worm burdens on day 14, again confirming earlier data and indicating that the wild strain was indeed infective.

The results of worm recoveries on day 70, i.e. 35 days after challenge infection with the laboratory strain (Table 4) show that a 6-day infection with both strains induced a strong immunity to reinfection with the laboratory strain (Mann-Whitney U-test, Group A versus Group B, $P < 0{\cdot}05$; Group A versus Group C, $P < 0{\cdot}001$). The strength of immunity induced by the heterologous (wild) strain was not significantly less than that induced by the homologous strain (Group B versus Group C, $P >$

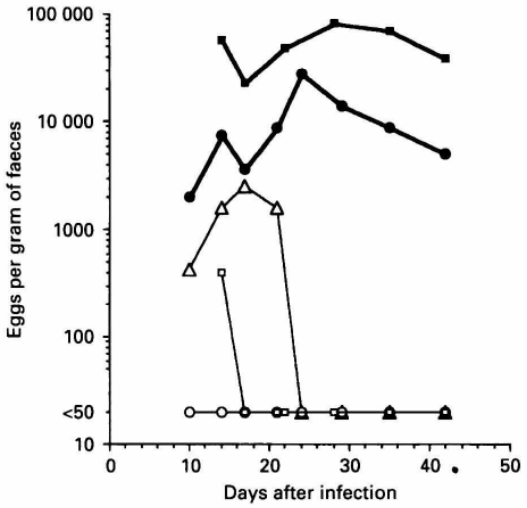

Fig. 1. Mean daily faecal egg production of CFLP mice, *Apodemus sylvaticus* or *Clethrionomys glareolus* infected with the laboratory strain of *Heligmosomoides polygyrus*. CFLP mice (●), *A. sylvaticus* (△) and *C. glareolus* (○) given 100 L_3 (Exp. 11); CFLP mice (■) and cortisone-treated *C. glareolus* (□) given 200 L_3 (Exp. 12). Note that < 50 eggs per gram of faeces could not be detected and this value therefore represents the limit of the sensitivity of the technique employed.

0·05), the slightly higher mean in Group B being attributable to one heavily infected mouse. Treatment after 27–28 days reduced the degree of immunity induced by infections with either strain, although the effect was much greater with the laboratory strain. Thus a 4-week infection with the laboratory strain did not elicit significant resistance, but a 4-week infection with the Stroud strain gave significant but weak immunity (Group A versus Group D, $P < 0·05$).

Survival and fecundity of H. polygyrus *in* Clethrionomys glareolus *and* Microtus agrestis

Table 5 summarizes the results of four experiments in which *C. glareolus* and *M. agrestis* were infected with the Oxford or laboratory strains (Exps 9–12). No worms of either strain were recovered at autopsy from either host species. The egg production of worms of the laboratory strain in *C. glareolus*, CFLP mice and *A. sylvaticus* is shown in Fig. 1 (Exp. 11). No eggs at all were recovered from *C. glareolus*, but there was a very brief period of egg production in *A. sylvaticus*, followed by a marked decline in the third week of infection. This again reinforces earlier data: a few parasites completed the tissue phase of development but their survival in the gut lumen was rapidly curtailed.

Cortisone treatment did not increase the survival of worms in *C. glareolus* (Table 5; Exp. 12), although some eggs were produced on day 14 in one animal (Fig. 1; Exp. 12) indicating that at least two worms developed to maturity before explusion.

DISCUSSION

It is important to emphasize from the outset that some of our experiments were carried out on wild-caught rodents of uncertain age, which would almost certainly have experienced the wild strain *H. p. polygyrus* before capture and would have been infected with a variety of other parasites as well. All the nematodes and ectoparasites would have been removed by the anthelminitic treatment, prior to laboratory infection. For obvious reasons we did not wish to use more wild-caught animals than were absolutely necessary and we were constrained by the density of local populations in particular years. Nevertheless, with these reservations in mind, it is quite clear from the results described in this paper that the wild strain of *H. polygyrus* (*H. p. polygyrus*) which infects *A. sylvaticus* in the U.K. shows quite different infection characteristics to the laboratory strain (*H. p. bakeri*). Very few wild-strain parasites from the three localities in the U.K. survived the early tissue stage of development in laboratory mice, and the few which did were rejected by NIH mice, but survived for longer in the weak responder C57BL/10 strain. It is probable that most of the larvae in the inoculum failed to become activated and did not establish, or were rapidly destroyed during the tissue phase of development in the *muscularis externa*. In contrast, the wild strain established chronic infections in *A. sylvaticus* (Table 1, Exp. 2; see also Gregory, Keymer & Clarke, 1990), despite the likelihood that the wild rodents would have experienced infection before capture.

The laboratory strain was more resilient; wild-caught *A. sylvaticus* developed comparatively heavy worm burdens at day 14 after infection with the laboratory strain (Table 1 and Table 3, Exp. 7). However, adult worms (laboratory strain) were expelled from *A. sylvaticus* in the weeks following patency, but not from laboratory mice. Thus resistance against wild-strain parasites in laboratory mice appears to be primarily directed against larvae, whilst that against laboratory-strain parasites in *A. sylvaticus* seems to be against adult worms.

In both combinations treatment with cortisone greatly enhanced susceptibility of the mice to infection. All laboratory mouse strains became susceptible to infection with the wild strain of larvae, and worm burdens as high as those observed in *A. sylvaticus* were recovered at autopsy. Similarly, *A. sylvaticus* tolerated heavy worm burdens of the laboratory strain for at least 4 weeks post-infection when treated with cortisone. These results, together with the data on worm fecundity, suggest that wild

and laboratory strains experience an environment which is suitable for their development in the heterologous host combination, but normally fail to develop because of immunological resistance, rather than any innate differences in host physiology. Those few worms which do develop have normal egg production. In contrast, *C. glareolus* and *M. agrestis* were totally insusceptible to infection with either strain of parasite, and cortisone treatment had no effect.

A similar range of host–parasite interactions has been shown with infections of the laboratory strain of *H. polygyrus* in other laboratory hosts. In laboratory rats very few worms develop, with the majority being encapsulated and killed in the intestinal wall; cortisone treatment prevents this cellular reaction and allows worm development (Cross, 1960). Cross (1960) also found that cortisone treatment was only effective before day 7 post-infection. Guinea pigs (*Cavia porcellus*) and golden hamsters (*Mesocricetes auratus*) are similarly refractory to infection (Newton, Weinstein & Jones, 1959; Cross & Duffy, 1963); in contrast, infections in jirds (*Meriones unguiculatus*) develop to adults, but are expelled from the host by week 5 (Jenkins, 1977; Hannah & Behnke, 1982).

The results presented here suggest that the host range of *H. p. polygyrus* in Britain is controlled by the resistance of different host species to infection, rather than behavioural or exposure-related factors. *H. polygyrus* has been reported from *Mus musculus* in Britain (Fahmy, 1956; James, 1954), but figures for prevalence or intensity are not available. Moreover, some studies of wild house-mice have failed to detect the species (Behnke & Wakelin, 1973; Behnke, 1975), whereas the majority of studies of *A. sylvaticus* have reported infection with *H. polygyrus*. It is possible that the few reports of *H. polygyrus* in British house-mice reflect infections in less immunocompetent hosts, and/or represent cross-infection from *A. sylvaticus*. Alternatively a *Mus*-adapted strain or subspecies may be responsible for these observations, as has been reported in North America (Whitaker, 1970). Forrester (1971) and Forrester & Neilson (1973) investigated the host specificity of laboratory and wild strains of *H. p. bakeri* in a range of wild North American mammals. Infections in the wild were present in *Mus* and *Reithrodontomys*, at prevalences of 25–40% and 4–14% respectively, while *Peromyscus maniculatus*, *P. leucopus* and *Microtus* sp. were uninfected. Laboratory infections in these species showed similar results: both wild and laboratory parasites successfully infected wild and laboratory *Mus*, and a wild strain infected *Reithrodontomys*, but the two species of *Peromyscus* and *Microtus* were refractory to infection unless treated with immunosuppressive agents (Helper & Leuker, 1976). However, a third *Peromyscus* sp., *P. eremicus*, which had been reported to carry infections in the wild (Babero & Matthias, 1967), was susceptible to experimental infection (Forrester & Neilson, 1973).

Reports of *H. polygyrus* from *Clethrionomys* and *Microtus* in Britain are more surprising, in view of the failure of our attempts to infect voles, including immunosuppressed animals, with either field or laboratory strains. A number of British studies have reported *H. polygyrus* from *C. glareolus* (Sharpe, 1964; Lewis, 1968*b*; Rainbow, 1971; Canning *et al.* 1973), or from *M. agrestis* (Elton *et al.* 1931; James, 1954; Lewis, 1968*b*; Lewis & Twigg, 1972). It is possible that genetic variation between hosts and/or parasites in the field allows infection; however, it is perhaps more likely that there has been some confusion with other, superficially similar, species of *Heligmosomoides*. For instance, *H. glareoli* has been reported from *C. glareolus* in Oxford (Baylis, 1928, 1939; Elton *et al.* 1931) and from both *C. glareolus* and *A. sylvaticus* in the Hebrides (Thomas, 1953). Additionally, voles in Europe are reported to be infected by several closely related species, whose taxonomy is still a matter of debate, such as *Heligmosomum* spp., *Boreostrongylus* spp. (see Thomas, 1953; Furmaga, 1957; Kisielewska, 1970; Durette-Desset *et al.* 1972, 1985; Tenora, Quentin & Durette-Desset, 1974; Tenora & Meszaros, 1971). In the USA a range of species have been reported from various voles (*Microtus*), including *Heligmosomoides bullosus*, *H. montanus*, *H. wisconsinensis*, *H. carolinensis*, *H. longispiculatus*, *Heligmosomum nearcticum* and *H. microti* (Dirkmans, 1940; Kuns & Rausch, 1950; Kinsella, 1967; Durette-Desset, 1968*b*).

Finally, an experiment was carried out to determine whether the two strains of *H. polygyrus* were capable of generating cross-resistance to each other. NIH mice were immunized by a short 6-day infection or by a longer 4-week infection. At the dose employed ivermectin was totally effective at killing all tissue larvae on day 6 post-infection (Wahid, Behnke & Conway, 1989), and it is evident from Table 4 that after a 6-day infection both strains were equally effective in inducing a protective immunity against subsequent challenge with laboratory strain larvae. The 4-week immunizing infections were less immunogenic, confirming earlier work by Behnke & Robinson (1985). However, the wild strain generated some degree of resistance while the laboratory strain did not. It is believed that the laboratory strain survives partially through immunomodulatory activity which protects adult worms from host resistance (reviewed by Behnke, 1987). The present results are consistent with this hypothesis: in the heterologous combination (i.e. NIH mice immunized with wild-strain larvae) the immunosuppressive activity of a 4-week primary infection would have been less intense, as few worms would have survived the tissue phase of development.

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