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Nematospiroides dubius in the jird, *Meriones unguiculatus*: factors affecting the course of a primary infection

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ABSTRACT

This project investigated the influence of the age and sex of the host and the level of infection with *Nematospiroides dubius* on the establishment and duration of a primary infection in the jird. It was found that 30 to 35% fewer worms matured in jirds than in mice and that this proportion was unaffected by the level of infection or by the sex of the host. In contrast the age of the jird was found to be critically important in determining the number of adult worms recovered 14 days after infection. Thus, maximum susceptibility to infection was observed when jirds were 18 to 22 days old. In older jirds susceptibility declined until 30 days after birth and thereafter, when approximately 70% of the infective larvae matured (relative to the number of worms maturing in mice).

The duration of a primary infection in jirds was similar in both sexes but was influenced by the level of infection and by the age of the host at infection. Heavier infections (500 larvae) lasted about 10 days longer than low level infections, and expulsion of the parasite occurred even when the host was infected with only five larvae. The duration of a primary infection was markedly prolonged in neonatal jirds, faecal egg counts remaining positive for 54 to 56 days after infection of 12-day-old jirds.

These results supply some further baseline data for future work on the biology of *N. dubius* in the jird and they provide support for the involvement of immunological processes in the termination of primary infections of this parasite in mature jirds.

INTRODUCTION

The jird, *Meriones unguiculatus*, is known to be susceptible to infection with the murine trichostrongyle *Nematospiroides dubius* (CROSS & SCOTT, 1960; CROSS, 1964; JENKINS, 1977). However, in contrast to the chronic survival of this parasite in laboratory mice, the adult worms are lost from the jird intestine within five weeks of infection. *N. dubius* infection in the jird therefore offers the opportunity of a comparative study which may help in the understanding of the mechanism by which this parasite avoids the protective responses of its normal host, the mouse.

The loss of worms from the jird resembles the immune rejection of other intestinal nematode parasites, such as *Nippostrongylus brasiliensis* and *Strongyloides ratti* from the rat, and *Trichinella spiralis* and *Trichuris muris* from the mouse. However, it is still uncertain if the elimination of worms from the jird is immunologically mediated (CROSS, 1964; JENKINS, 1977; HANNAH & BEHNKE, 1982). Therefore, in the present work we have investigated the natural parameters that affect a primary infection of *Nematospiroides dubius* in the jird as a preliminary to further analysis of the factors which prevent the parasite's survival in this host beyond five weeks after infection.

MATERIALS AND METHODS

Nematospiroides dubius

The origin and maintenance of our strain of *N. dubius* and the methods used for infection and recovery of adult worms have already been described (BEHNKE & WAKELIN, 1977; BEHNKE & PARISH, 1979).

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Faecal egg counts

One gram of faeces was taken from the pooled faeces of all the animals in each group, deposited over the preceding 24 hours, and was dispersed in 8 ml of 50% saturated saline. This suspension was washed through a sieve (aperture size 800 μm) with 35% zinc sulphate solution and the eggs were counted after flotation in standard McMaster counting slides as described by GORDON & WHITLOCK (1939). The counts are expressed as the number of eggs per gram of whole faeces.

This method used a large proportion of the faeces deposited in 24 hours by each experimental group, and each sample was counted six times to ensure accuracy. In the groups in which faecal egg counts became negative three additional counts were carried out to ensure that the production of eggs had ceased. In preliminary experiments it was found that animals killed after egg counts became negative did not harbour any worms, and so the last day on which eggs were detected in the faeces is assumed to be the last day of infection.

Animals

Random bred jirds and CFLP mice were used throughout this work. The animals were bred and maintained under conventional animal house conditions in the Zoology Department of Nottingham University. Unless otherwise stated, the minimum age of animals used for experiments was eight weeks for jirds and five weeks for mice.

Statistical Analysis

Differences in adult worm recoveries were analysed for significance by the non-parametric Wilcoxon test (SOKAL & ROHLF, 1969). Straight lines were fitted to data by the method of least squares and correlation coefficients are indicated by r.

A value of $P<0{\cdot}05$ was considered to be significant for a comparison of worm recoveries, and the 'goodness' of fit of a line to the data.

RESULTS

Worm burden and egg production of N. dubius *in jirds*

Two experiments were carried out in order to confirm in our laboratory, the course of a primary infection in jirds. Fig. 1 shows the faecal egg counts from experiment 1 (Expt 1) in which five male jirds and five female mice were each infected with 150 *N. dubius* larvae.

In Expt 2, 12 female jirds and 24 male mice were each infected with 100 larvae, and groups of three jirds and six mice were killed on days 10, 15, 20 and 25 after infection. The mean worm recoveries (MWR) from these groups are shown in Fig. 2. On days 10 and 15 the MWR from jirds were 49·0 and 55·7 respectively, a level of infection which is significantly lower (30%) than the MWR from the mice (the latter being considered here as representing the maximum infectivity of the inoculum). This reduction in the number of worms recovered from jirds on days 10 and 15 indicates that approximately 30% of the administered infective larvae either failed to establish in this host, or alternatively, that the worms did not survive the tissue phase of their development.

Effect of the level of infection

In order to determine whether the lower MWR from jirds (compared with mice) were influenced by the number of larvae in the dose administered, the results of 30 separate experiments, in each of which the MWR from jirds could be directly compared with the MWR from mice, were analysed. The experiments covered worm burdens in mice ranging from 50 to 350, and the pooled results are illustrated in Fig. 3. The MWR

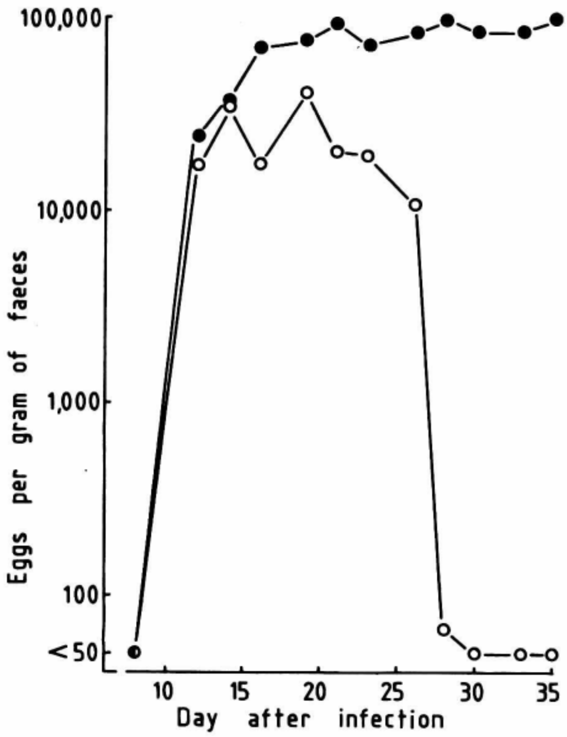

FIG. 1. Faecal egg counts from jirds (○) and mice (●) infected with 150 larvae of *N. dubius* (Expt 1).

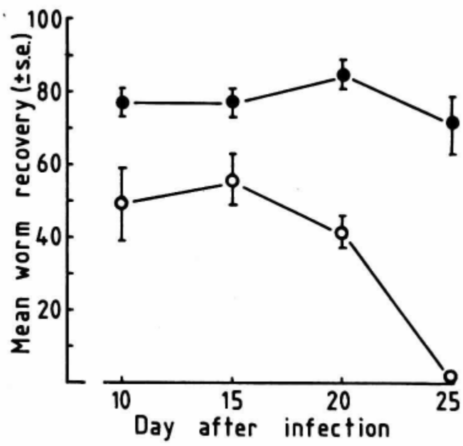

FIG. 2. Recovery of *N. dubius* from jirds (○) and mice (●) infected with 100 larvae (Expt 2).

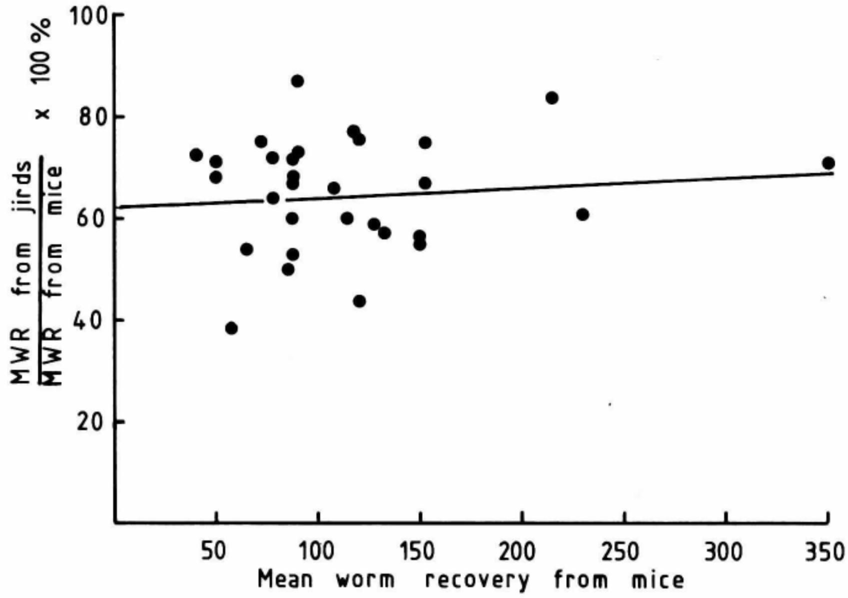

FIG. 3. Recovery of *N. dubius* from jirds (expressed as a percentage of the recovery from mice) given different numbers of infective larvae ($y = 62{\cdot}3 + 0{\cdot}02x$, $r = 0{\cdot}12$, $P > 0{\cdot}1$).

from mice are plotted against the MWR from jirds; the latter being expressed as a percentage of the MWR from mice. In jirds the percentage MWR ranged from 38 to 87% with an over-all mean ($\pm$ s.e.) of 64·8% ($\pm 2{\cdot}1$). A line fitted to these data is given by the equation $y = 62{\cdot}3 + 0{\cdot}02x$ ($r = 0{\cdot}12$), but since this equation does not closely fit the data ($P > 0{\cdot}1$) it would appear that the level of infection, as monitored by MWR in mice, does not influence the percentage of larvae which become established in jirds. Therefore, within the range of infection levels that were considered jirds would be expected to harbour approximately 35% fewer worms than identically infected mice, 10 to 20 days after a primary infection.

In the preliminary experiments reported in the previous section the duration of the infections with *N. dubius* was different. Thus, in Expt 1 eggs were observed in the faeces of infected jirds until day 28, whereas in Expt 2, jirds were already worm free on day 25. Expt 3 was designed to investigate if the survival time of *N. dubius* in jirds was related to the level of infection. Nine male jirds were divided equally into three groups, and each group was infected with 25, 150 or 500 larvae. Fig. 4 shows the faecal egg counts from all three groups over the next six weeks. The last observed day of egg production ranged from day 21 in the group given 25 larvae, to day 39 in the group given 500 larvae. This experiment was repeated with similar results suggesting that there is a positive relationship between the level and duration of infection.

To confirm this finding the results from another 34 experiments were analysed. The experiments covered doses of five to 500 larvae and the results are illustrated in Fig. 5, the duration of infection (defined as the last observed day of egg production) is plotted against the level of infection. All infections, including those of only five larvae, terminated within 41 days. A line fitted to the data, given by the equation $y = 23{\cdot}7 + 0{\cdot}026$ [illegible] is highly significant ($r = 0{\cdot}73$, $P < 0{\cdot}001$).

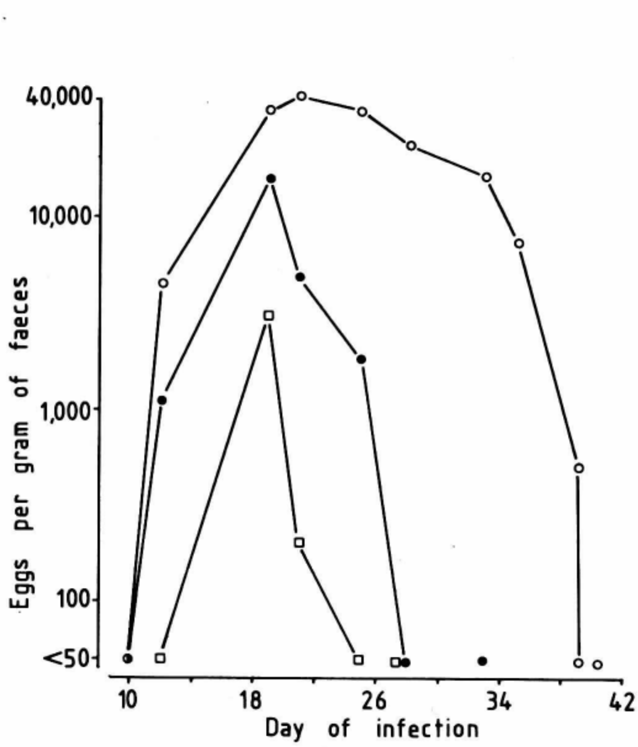

FIG. 4. Faecal egg counts from jirds infected with 25 (□), 150 (●) or 500 (○) larvae of *N. dubius* (Expt 3).

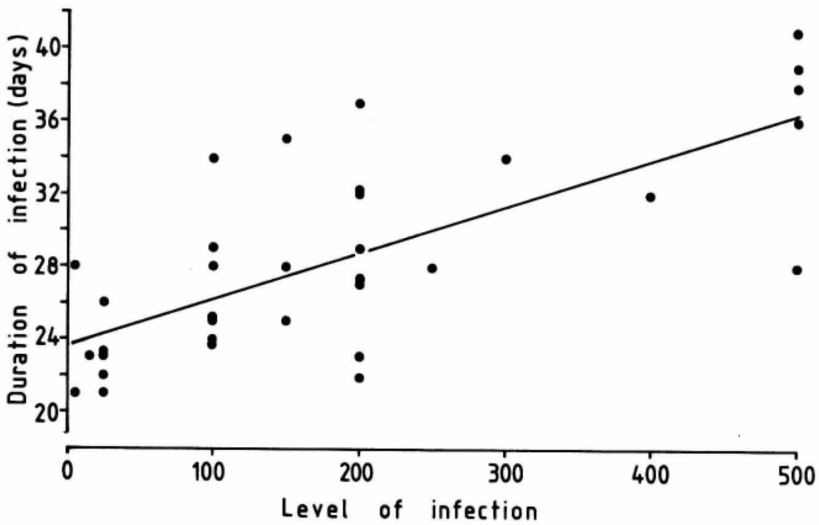

FIG. 5. Correlation between the duration and level of infection with *N. dubius*. Each point represents a group of at least three jirds ($y=23{\cdot}7+0{\cdot}026\times$, $r=0{\cdot}73$, $P<0{\cdot}001$).

Effect of host sex

In Expt 4, three male and four female jirds were each infected with 100 larvae, and all the animals were killed on day 16. The MWR ($\pm$s.e.) from the male jirds was 55·2 ($\pm$5·5) and that from the female jirds was 57·8 ($\pm$7·0). Since there is no significant difference between these MWR, it must be concluded that the sex of the host did not affect the establishment of *N. dubius*.

Table I gives the results of Expts 5 to 8 in which the duration of infection was compared in male and female jirds. In two of the experiments (Expts 7 and 8) the duration of infection was longer in the males, but in Expt 6 the duration was longer in the females, and in Expt 5 the duration was identical in both groups. These results indicate that in our experimental system there is no consistent difference between the duration of infection in male and female jirds.

TABLE I. Duration of *N. dubius* infection in male and female jirds (Expts 5 to 8). The number of animals in each group is given in parentheses.

Experiment	No. of Larvae	Last observed day of egg production	
		Male jirds	Female jirds
5	150	25(3)	25(3)
6	150	28(5)	35(5)
7	100	27(4)	23(4)
8	25	26(5)	23(5)

Effect of host age

Expts 9 to 11 investigated the establishment of *N. dubius* in jirds of different ages. In each experiment several groups of jirds and a control group of mice were all infected with 100 larvae, and killed 14 days later. Fig. 6 shows the MWR from the jirds in these three experiments (expressed as a percentage of the MWR from the control mice), each point is based on three to 12 animals. These results show that jirds are increasingly susceptible to *N. dubius* infection from 10 days up to 20 days of age. However, this susceptibility then declines reaching a consistent level at 30 days of age and thereafter.

Fig. 7 shows the results of Expt 12 in which faecal egg counts were carried out on groups of jirds (five animals per group) which were infected with 200 larvae of *N. dubius* when 12 days, three or six months old. The duration of infection was found to be greatly prolonged in neonatal jirds when compared with their mature controls. This experiment was repeated and the results were almost identical; the duration of infection in mature animals was 32 days and in neonatal jirds (13 days old) 50 days.

DISCUSSION

The results presented in this paper can be conveniently discussed under two separate headings, the first section considering the factors which affect the establishment of *N. dubius* in jirds, and the second, the factors influencing the duration of a primary infection.

Establishment of N. dubius *in the jird*

The jird is susceptible to a primary infection with *N. dubius* but fewer worms reach maturity than in control mice. This reduced susceptibility is variable between experiments but, on average, 35% fewer worms are recovered from mature jirds than

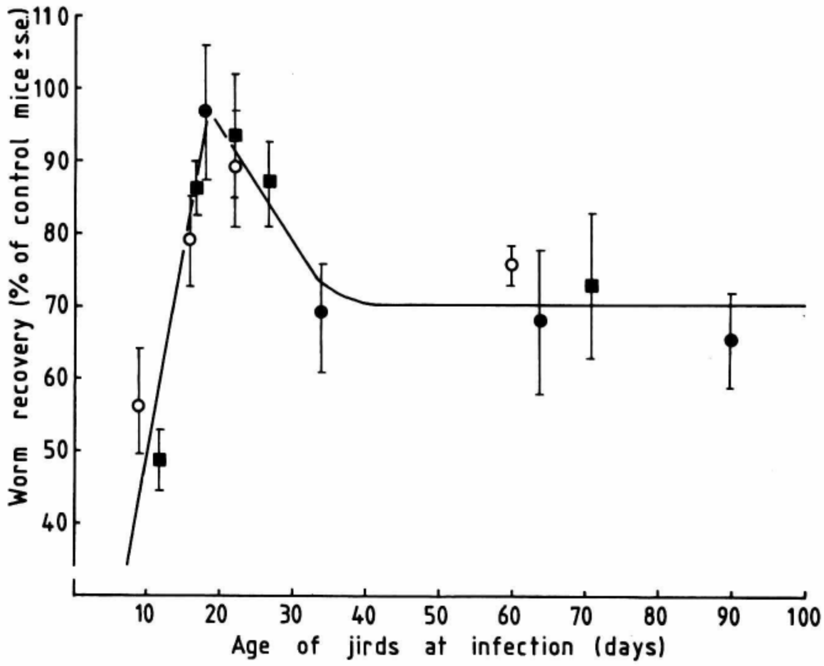

FIG. 6. Recovery of *N. dubius* from jirds of different ages, 14 days after infection with 100 infective larvae (○ = Expt 9, ● = Expt 10, ■ = Expt 11).

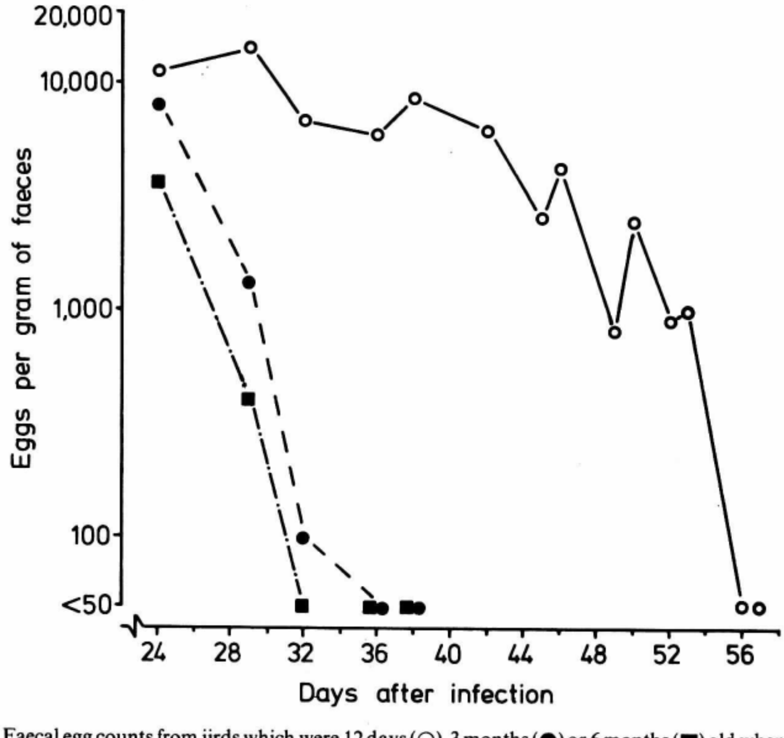

FIG. 7. Faecal egg counts from jirds which were 12 days (○), 3 months (●) or 6 months (■) old when infected with 200 larvae of *N. dubius* (Expt 12).

from mice. The present results also reveal that this reduction in the number of worms reaching maturity is independent of the level of infection. JENKINS (1977) found that male and female jirds harboured similar number of worms until day 21 and this finding has been confirmed by the present work.

The one factor that was found to affect the proportion of *N. dubius* reaching maturity was the age of the jirds when they were infected. This age related susceptibility exhibited three phases. In the first phase the jirds became increasingly susceptible until, at 20 days of age, the jirds harboured as many worms as control mice. SUKHEDO & CROLL (1981) showed that the concentration of bile in the intestine is an important factor in the establishment of *N. dubius* larvae in rats and mice, and they also suggested that other factors may be involved. Thus, the increasing susceptibility of young jirds with age may be the result of corresponding changes in the environmental conditions of the intestine, until at 20 days of age the environment is suitable for the maximum establishment of *N. dubius* larvae.

The second phase was seen in animals 20 to 30 days old at infection. Between these ages the MWR fell from 96% to 70% of the MWR from control mice. This increasing resistance related to age has previously been reported for *N. dubius* infection in another abnormal host. CROSS & DUFFY (1963) showed that rats become increasingly refractory to infection up to the age of five weeks, when they were essentially resistant. CROSS (1960) and CROSS & DUFFY (1963) showed that the resistance of adult rats to *N. dubius* is mediated by a cellular reaction which results in the encapsulation of the larvae in the intestinal tissue, and that this reaction is minimal in immature animals. It is therefore conceivable that the increasing resistance of jirds between 20 and 30 days of age to *N. dubius* is a result of the development of an inflammatory and/or a cellular response which traps a proportion of the developing larvae.

The final phase in the age related susceptibility of jirds was seen in animals over 30 days old at infection. The number of worms recovered from such animals was approximately 70% of that from control mice. Therefore, in marked contrast to the rat which becomes totally refractory to infection, the jird retains its susceptibility but at a level which corresponds to 65 to 70% of the infectivity of the inoculum, and this may reflect the maximum efficiency of the inflammatory response which, nevertheless, allows most of the worms to reach maturity.

Duration of N. dubius *infection in the jird*

The duration of infection in individual jirds given the same inoculum is known to be variable, CROSS & SCOTT (1960) found that faecal egg counts became negative 21 to 32 days after infection. However, our results indicate that the level of infection is also important in determining the length of an infection, and Fig. 4 shows that *N. dubius* survived in jirds given 500 larvae for 10 days longer than in animals given only 25 larvae. The prolonged survival of *N. dubius* in heavily infected jirds could be due to stress and pathogenesis resulting from harbouring a heavy worm burden, this perhaps impairing or delaying the mechanism of parasite expulsion. However, our animals did not appear to be unduly stressed and there was no mortality, the levels of infection being well below the lethal level for jirds (personal observation). An alternative or additional explanation could be that there is a dose-related suppressive effect upon worm expulsion. There is evidence, in mice, to indicate that *N. dubius* affects the host's ability to generate and express anti-parasite immune responses in the intestine (BEHNKE *et al*., 1978; HAGAN & WAKELIN, 1982) and although this may be less pronounced in the jird, nevertheless, the immune response would be more severely depressed in heavily infected animals.

JENKINS (1977) reported that the loss of worms from female jirds took place earlier than in males. In the present study there was no evidence to confirm this observation, expulsion in both sexes occurring almost simultaneously (Expts 5 to 8). This inconsistency could be explained in terms of differences between the jird strains, resulting from inbreeding of the stock animals in the laboratories concerned.

The greatest alteration to the normal course of an infection with *N. dubius* in jirds was found in neonatal animals. The duration of infection in jirds infected when 12 days old was about eight weeks, which is considerably longer than in any other experiment thus far reported for *N. dubius* in this abnormal host. It is not altogether an unexpected result since the rejection of *Nippostrongylus brasiliensis* from neonatal rats (JARRETT *et al.*, 1966, 1968; KASSAI & AITKEN, 1967; OGILVIE & JONES, 1967) is also delayed and is thought to be caused by the slow maturation of the cellular component of the processes which lead to worm expulsion (DINEEN & KELLY, 1973; OGILVIE & LOVE, 1974), and may possibly involve enhanced suppressor cell activity (CALKINS & STUTMAN, 1978). The similarity between the pattern of expulsion of *Nematospiroides dubius* from jirds and *Nippostrongylus brasiliensis* from rats, and the prolonged survival of both species in their neonatal hosts argues for the involvement of immunological processes in the termination of *Nematospiroides dubius* infections in mature jirds.

Finally, the experiments reported in this paper have considered the importance and inter-relationship of the three most important natural factors (age and sex of host, infection level) which may influence the establishment of a parasite in its host and the course of the subsequent infection. It is clear that both age of the host and the intensity of infection can have a marked influence on the outcome of an experimentally administered infection and will therefore need to be carefully considered in future studies. This work has provided baseline data which will be of value in designing future experiments attempting to elucidate precisely how *N. dubius* infections are terminated in jirds. The fact that the adult parasite can be successfully expelled from the jird intestine means that this organism is susceptible to an immune response in the intestine of a rodent, it is therefore all the more intriguing that such a response does not occur against *N. dubius* in the mouse.

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