DYNAMICS OF REPEATED INFECTION OF HIGH AND LOW RESPONDER INBRED MICE WITH *HELIGMOSOMOIDES POLYGYRUS*

by

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B.Sc.(Lesotho), M.Phil (Cantab.)

Thesis submitted for the degree of Doctor of Philosophy and Diploma of Imperial College in the Faculty of Science of the University of London.

October 1986

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ABSTRACT

The population dynamics of repeated infection of laboratory mice with the hookworm *Heligmosomoides polygyrus* is investigated. The heritability of the innate host response to *H.polygyrus* is assessed by the selection of high and low responders from an outbred mouse population.

Single infection experiments are employed to obtain quantitative estimates of various population rate-determining parameters. Parasite establishment was linearly related to the density of infective larvae. Parasite fecundity was found to be both age- and density-dependent, and parasite mortality was only adversely affected at very high parasite burdens per host.

Under conditions of repeated exposure to infection, parasite population growth increased monotonically to a plateau in low responder mice. However, temporal changes in mean worm burdens in high responder mice exposed to a high dose of infection revealed a convex pattern. A mathematical model which incorporates a reduction in parasite establishment and increased parasite mortality rate due to an accumulated experience of past infection, is employed to analyse and interpret the data.

Antibody responses to crude and surface antigens of the parasite are compared between the high and low responder mouse strains during repeated infection, using ELISA, immunoprecipitation, SDS-PAGE and autoradiography. The impact of chemotherapy and vaccination on the dynamics of repeated infection, and the duration of acquired resistance in these mice, are assessed. The results are discussed in the light of current knowledge of the epidemiology and control of intestinal nematode infections of humans.

ACKNOWLEDGEMENTS

I am grateful to the Commonwealth Commission (UK) for the award of an Academic Staff Scholarship.

I am very much indebted to my supervisor, Professor Roy Anderson F.R.S., for being a pillar of constant support, encouragement and inspiration during the course of this project. I thank him for improving the presentation of this thesis, although of course, I am entirely responsible for any mistakes or errors in this work. I thank Dr. John Lewis for parting with the original stock of the parasite, and Dr. J.M. Behnke, for providing the irradiated larvae.

I also thank Dr. Rick Maizels, Chris Langford and Graham Medley for helpful advice and comments on some of the chapters. I have benefitted greatly from discussions with Graham Medley and Jenny Crombie on the mathematical models, and also from several discussions and help from the other members of the PERG, including my colleague, Paiboon Sithithaworn. Brian Robertson, Rick Maizels and Mike Morgan guided me through the immunology techniques. The technical assistance of Mr. Paul Morriss and staff of the Animal Unit: Steve, Gary, Susan and Christine and John, is gratefully acknowledged. Ian French and Anne Mary were helpful in several ways.

Finally, I would like to thank all our friends for contributing in their own special way towards making life easier for my wife and I in Britain. Kekeletso and Itumeleng Mosala have supported us in many ways and Puleng Mokaeane has always been around at the right time. My parents and my wife's parents have always made us feel wanted, despite the physical distance between us. To my wife, Lisebo, and our two daughters, Nthakoana and Ntema: thank you for being so patient - you are very special to me.
I dedicate this thesis to my deceased parents, Mr and Mrs A.J. Maema, who passed away while I was in Britain -
I am sure you would approve.....

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Chapter 1

General Introduction

1.1 Historical background

The hookworm Heligmosomoides polygyrus Dujardin,1845 (=Nematospiroides dubius Baylis,1926)is a natural parasite of wild rodents found in North America,Western Europe and the USSR(Kuns & Rausch,1950; Rausch,1952; Yamaguti,1961; Forrester,1971; Durette-Desset et al,1972). Since its first introduction into the laboratory by Spurlock(1943), this parasite has been extensively used as an experimental model.

Its taxonomy has, however, generated much controversy. The shift of popular acceptance has been towards the name H.polygyrus rather than N.dubius (Durette-Desset,1985), although the debate surrounding its systematic position continues. Dujardin(1845) first described the parasite from field mice, Mus(Apodemus) sylvaticus, and the voles, Arvicola(Microtus) arvalis, in France, and named it Strongylus polygyrus. Unfortunately, he neither adequately described the male bursa- a character which is used to distinguish species in this family of nematodes, nor preserved enough specimens to enable future analysis. Hall(1916), on the authority of Travassos(1914), re-classified the parasite as Viannaia polygyrus, yet at the same time, recognised a new genus, which he named Heligmosomoides. However, Boulenger(1922) argued that Hall's species was in fact similar to Schultz's and Dujardin's and re-classified S.polygyrus as

H.polygyrus in an attempt to reconcile Hall's and Dujardin's disparate views. Baylis(1926), using evidence from material collected from A.sylvaticus in Oxford,England, was of the opinion that Strongylus most closely resembled the Heligmosominae family, but felt that there was sufficient justification to erect, on the basis of the similarity between Walton's(1923) Nematospira and Hall's(1916) Heligmosomoides, a new genus, Nematospiroides and the species was called dubius, to reflect the doubtful nature of the parasite.

Classification based on morphological relationships between hosts and parasites can be misleading since both are co-evolving and laboratory studies have increasingly shown how the host-parasite interaction can influence such taxonomic considerations(Haley,1962). In any case, since N.dubius was brought into the laboratory 40 or more years ago (Spurlock,1943), this parasite has probably undergone some adaptation. Indeed, the infectivity of Mus musculus - laboratory passaged H.polygyrus was shown to have ablated in laboratory-reared Peromyscus maniculatus gambeli, although it was isolated from a wild P.m.gambeli in 1950 (Forrester & McL Neilson,1973). Nevertheless, such taxonomic controversies need not detract from the usefulness of this parasite as a model of chronic hookworm infection in man (Bartlett & Ball,1972).

1.2 Life history

H.polygyrus is a direct life-cycle trichostrongylid found in microtine rodents(Fig.1.1). The parasitic stage is in the rodent and the free-living stages persist in the host's environment. The life cycle was first described in full by Fahmy(1956) when he successfully infected laboratory mice with H.polygyrus collected from wild A.sylvaticus. Before then, Elton et al(1931) had observed small cysts in the external muscularis mucosa of wild mice as well as larvae on their skin. They attributed the presence of adult H.polygyrus in the lumen of the small intestine of mice to transmission via skin penetration. Roman(1951),however, disputed the purported ability of infective larvae to penetrate mouse skin, and thought that infection was by oral ingestion of larvae. In the present study it has not proved possible to infect mice percutaneously with H.polygyrus using the ring-method technique of Smithers & Terry(1965). Investigations into the life-cycle of H.polygyrus by Ehrenford(1954), Fahmy(1956), Lewis(1968a) and Bryant(1973), have confirmed the general life history of this parasite although slight differences in the timing of particular events exist.

Adult male and female H.polygyrus are selectively found in the proximal part of the small intestine (Lewis & Bryant,1976; Sukhdeo & Mettrick,1983; Sukhdeo & Croll,1981) where they are closely associated with the villi and epithelium, and bathe freely in the lumen. Following mating, mature females lay

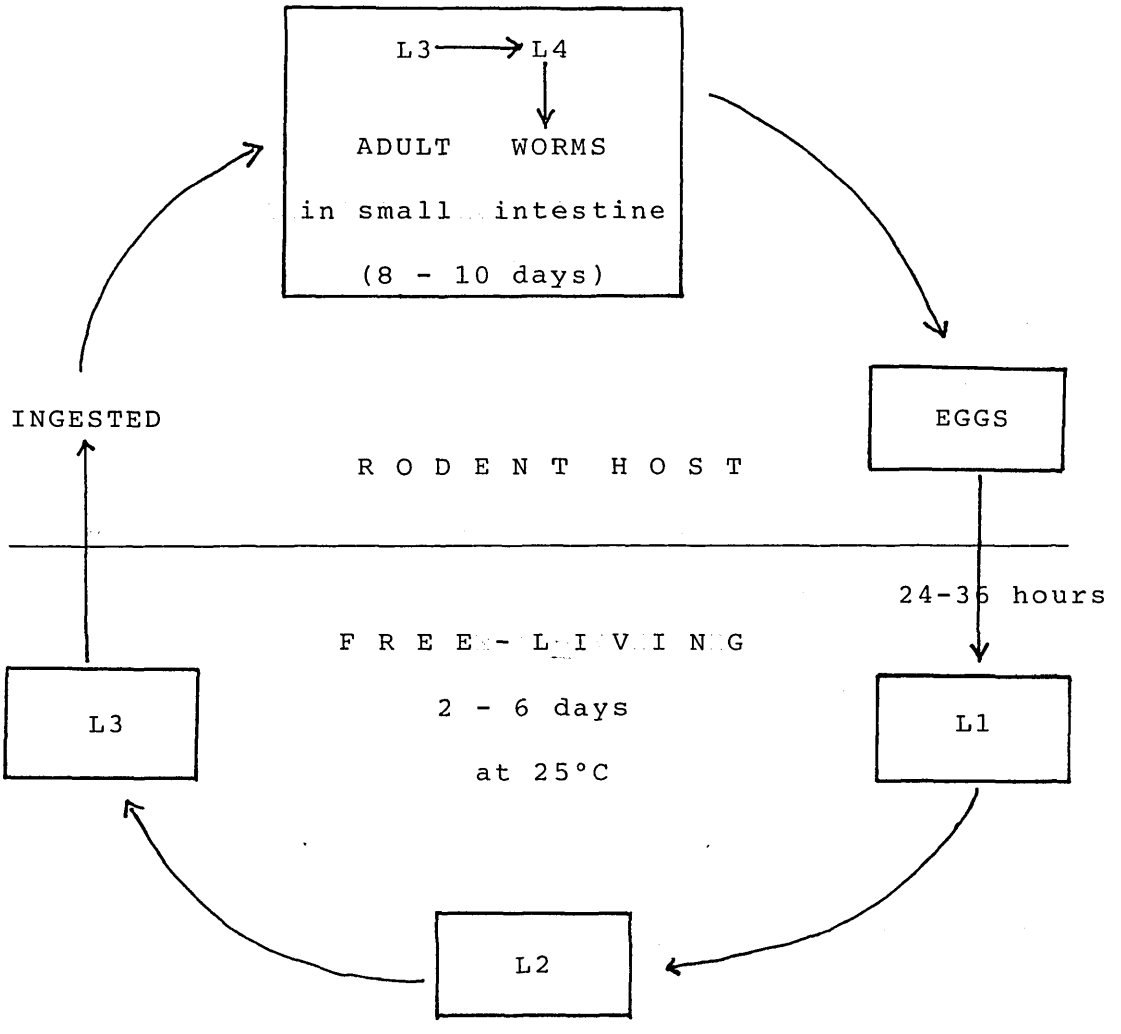


Figure 1.1. The life cycle of Heligmosomoides polygyrus (= Nematospiroides dubius).

fertile eggs that are excreted with host faeces into the environment. Under suitable environmental conditions (Kerboeuf,1978; Yasuraoka & Weinstein,1969; Bryant,1973), eggs hatch within 24-36h and develop into first stage(L1) and second stage(L2) larvae which feed on micro-organisms. Between 2-6 days, L2 undergo a further moult to become ensheathed third stage(L3) infective larvae, which will remain in the environment awaiting ingestion by a susceptible host. Upon ingestion, intestinal stimuli initiate ex-sheathing of the L3 which then penetrates the muscle walls and remains in the mucosa, following a further moult to the fourth stage(L4) larvae. Six to eight days later, pre-adult stages appear into the lumen and the first eggs appear 8-10 days after infection. Thus, there are distinct tissue and luminal phases in the life cycle of H.polygyrus. No complex migration occurs in contrast to the life cycle of the human hookworms (Necator americanus, Ancylostoma duodenale). The L4, perhaps by virtue of their close contact with host tissue, are thought to account for the immunity observed in hosts infected with H.polygyrus.

1.3 Natural infections in wild rodents

Surprisingly few detailed longitudinal surveys of helminths in wild rodents have been published to date. Longitudinal studies of prevalence and intensity of infection are preferable over horizontal surveys in the sense that long-term changes in the prevalence and intensity of infection can be monitored

in the host community (Anderson & May,1985a). Published epidemiological surveys of helminth parasites in England and Wales and North America, cover study periods ranging from 1-2 years and have involved trapping of over 200 animals, include those of Elton et al(1931), Thomas(1953), Sharpe(1964), Lewis(1968a,b), Forrester(1971), Rainbow(1972), Lewis & Twigg(1972), Murua(1978) and Langley & Fairley(1982).

In general, a high and steady prevalence but fluctuating intensity of H.polygyrus infection have been observed. The intensity is generally high during the Spring-Summer season and low in Winter(Murua,1978). In North Carolina, Forrester(1971) found an overall prevalence of only 9% although in M.musculus, it was as high as 25%, with a mean intensity of 5.8 worms/mouse. Sharpe(1964) reported a high prevalence and mean intensity of 29.5 worms/mouse from A.sylvaticus and Clethrionomys glareolus from Bristol. Similarly, Murua(1978), also studying A.sylvaticus and Cl.glareolus populations from open oak woodland in Bristol, reported 95-98% prevalence with a mean of 20 worms/mouse. In Oxford, Elton et al(1931) found a 60% prevalence and average worm burdens of 8.5-27.5 worms/mouse from A.sylvaticus of different age.

Lewis(1968a) and Lewis & Twigg(1972) provided details of mean worm burdens as a function of sex and age of host and estimated the number of immature H.polygyrus recovered from mature and immature mice as probable indicators of the rate of infection. Prevalence was between 50-95% and the

parasite burden was 12.0-24.7 worms/mouse. Lewis(1968a) found that the frequency distribution of H.polygyrus per host could be described by a negative binomial probability model, that is, few mice harboured most of the worms while many had no or few worms(the variance was greater than the mean). He attributed the generation of this overdispersed distribution to the way in which infective stages were aggregated. This hypothesis of a spatially heterogenous distribution of infective stages was not tested experimentally. Overdispersion in worms per host can arise even in uniformly distributed infective stages since successive random invasions of parasites of differing severities through time can generate negative binomial distributions (Keymer & Anderson,1979; Anderson et al,1978; Crofton,1971; Anderson & Gordon,1982).

Lewis(1968a) also found that male A.sylvaticus had more worms than female mice. Although hormonal sex differences have been described(Dobson,1961), Lewis was of the opinion that wider ranges and greater foraging activity by males(Smal & Fairley,1982) generated a greater chance of picking up infective H.polygyrus among male hosts.

An epidemiologically important point made by Lewis (1968a) and also by Elton et al(1931), was that there were more adult than larval worms, and adult mice became more heavily infected than juveniles i.e. worm burden and age distribution of parasites in mice were dependent on mouse

age. Thus, a gradual accumulation of worms in the presence of minimal host resistance led to high worm burdens in the adult age-classes of the mouse population. Alternatively, owing to the observed longevity of H.polygyrus in laboratory mice, the absence of larvae in adult mice could be indicative of acquired resistance which acts to decrease larval establishment in mice already harbouring adult infections.

The most important points to emerge from studies of H.polygyrus in its natural hosts are that the prevalence is high and stable over long periods of time, but the intensity is low and fluctuates seasonally. The patterns can best be explained from a knowledge of the life history of the parasite in relation to transmission. They reflect a balance between long-lived adult worms and regulation of the parasite populations by exposure to infection and acquisition of resistance to re-infection. Testable hypotheses can be generated from these observed epidemiological trends. For example, what would be the effect of varying the exposure of mice to parasites? What would be the outcome of a repeated low-level infection in mice? Is there acquired resistance to H.polygyrus in mice and if so, are certain hosts 'better' transmitters of the parasite than others?

To date, few studies have addressed these issues despite their obvious importance to an understanding of

the parasite's transmission dynamics and ecology(Anderson & Crombie,1985; Healing & Nowell,1985; Keymer,1985; Scott,1985). Slater & Keymer(1986) have established natural H.polygyrus infections in CD1 mice in the laboratory by introducing a few infected mice into cohorts of uninfected mice in defined arenas and then monitoring the spread of infection with time. Scott(1986) has also established natural infections of H.polygyrus in large cages of breeding CD1 mice. Under these conditions, H.polygyrus was shown to induce significant mortality in the breeding colony. Experiments are now in progress to assess whether the remaining mice, following catastrophic mortalities in the colony, have been selected for refractoriness or not (Scott, personal communication). Such catastrophic mortalities are unlikely to occur in natural habitats as mice tend to disperse(Butler,1980; Lidicker,1975; Wolton & Flowerdew,1985) and live in small family units dominated by a single individual (Reimer & Petras,1967; DeFries & McClearn,1970). Moreover, other environmental selection pressures such as weather, predators and resources may be more efficient at controlling mouse populations than the parasite itself. Macroparasites are thought to be less likely to be major regulatory factors of their host populations (Anderson,1982a).

1.4 Laboratory infections

Information on experimental infection of laboratory animals with H.polygyrus is widely available. Natural resistance to infection has also been described in abnormal hosts. Cross & Duffy(1963) found reduced parasite survivorship and fecundity in rats. The jird, Meriones unguiculatus, is also susceptible to infection and worms become patent for a short period(Cross & Scott, 1960; Jenkins,1977) with fewer worms reaching maturity than in the mouse host (Cross & Scott,1960; Hannah & Behnke,1982). The jird is, however, highly sensitive to immunization with H.polygyrus (Behnke & Hannah,1984). Thus, there appears to be a substantial degree of host-specificity in H.polygyrus infections.

Natural resistance to H.polygyrus in mice is thought to be under genetic control. Liu(1966a), using the criteria: parasite-induced host mortality, worm burdens following primary infection and infectivity and survivorship of worms, has categorised the inbred homozygous C3H mouse strain as more susceptible to infection than the A, CF1, C57 and outbred Webster strains. Spurlock(1943) classified several strains into susceptible and resistant phenotypes on the basis of the host mortality induced by the parasite. One suggested mechanism for the observed high susceptibility of C3H mouse strain to H.polygyrus was thought to be a congenital defect in tissue resistance to

larvae, exacebated by poor cytopoiesis of the reticulo-endothelial system (Liu,1966a).

Host sex effects in the relationship between laboratory mice and H.polygyrus have been discussed by Dobson & Owen(1978). Dobson(1961) observed a reduced resistance to H.polygyrus during primary infections in male compared to female mice and attributed the increased resistance in female mice to the presence of oestrogen hormones. Changes in hormonal balance induced during pregnancy and lactation render mice more susceptible to parasites (Dineen & Kelly,1972; Behnke & Wakelin,1973; Connan,1970;1972; Wakelin,1984) and this adaptation is thought to be of some relevance to the survival strategy of parasites(Wakelin,1984). However, the role played by female hormones is controversial (Healing & Nowell,1985).

Host age and sex differences in susceptibility or resistance to H.polygyrus are less well documented in primary than in secondary or challenge infections (Hosier & Feller,1973; Hosier,1974; Prowse et al,1979). Cypess, Van Zandt & Zidian(1973) did not observe a consistent relationship between susceptibility and sex of outbred S-W mice over a period of 50 weeks. However, they reported that young mice (<4weeks old) did not demostrate acquired resistance following repeated infections. Further work needs to be done to establish whether or not mouse-sex differences in susceptibility or resistance to H.polygyrus are in fact physiologically mediated or purely artifacts of the differences in sizes of both male and female mice.

Acquired resistance has been measured using several parameters (Prowse & Mitchell,1980; Wakelin,1982). In general, one or more of the following traits has been used to characterize the resistance status of a host with respect to H.polygyrus: worm burden, growth, survivorship, fecundity and expulsion; inhibition of larval establishment and/or maturation; parasite-induced host mortality and immunological criteria such as the ratio of encysted larvae to granulomata in the intestinal wall(Chaicumpa et al,1978; Prowse & Mitchell,1980) and also, the ability to respond to adoptive transfer of cells and/or serum (Wakelin,1985).

The availability of inbred mouse strains, their recombinants and hybrids (Wakelin,1982; Festing,1982) has greatly facilitated our understanding of the role of host genotype in the control of helminth infections. Behnke & Wakelin(1977) have characterized, using a complex immunizing schedule, three inbred mouse strains. Balb/c were observed to be of intermediate resistance between the highly resistant NIH and the least resistant C3H mouse strains (Behnke & Wakelin,1977). Outbred LACA mice and inbred CBA/H and C57BL/6 mice were less resistant than inbred Balb/c, DBA/2, SJL/J and female C3H/HeJ mice (Prowse et al,1979). CBA/H male mice were found to be susceptible whereas young female Balb/c were resistant (Mitchell & Prowse,1979; Hurley et al,1980). The NZB

inbred mouse strain was killed by the immunizing infection, probably due to its hypersensitivity and propensity to intestinal haemorrhage (Mitchell & Prowse,1979; Prowse & Mitchell,1980). Moreover, it was observed that the progeny of the highly resistant SJL/J and the susceptible C57BL/6 inbred mouse strains were resistant (Prowse & Mitchell,1980). In fact, (C57BL/6 x SJL/J)F1 male mice were relatively less resistant than their female littermates, implying that resistance was inherited in a dominant fashion, but only expressed in female mice (Prowse & Mitchell,1980). Mouse sex differences in the inheritance of resistance or susceptibility to H.polygyrus is clearly an area for further research(Prowse & Mitchell,1980).

Mice which were selectively bred as high and low antibody responders to a variety of particulate antigens (Biozzi et al,1979) have been used by Jenkins & Carrington (1981) to investigate the course of H.polygyrus infections. Following multiple (but not primary) infections, the high antibody responder mice were found to harbour significantly less worms than the low antibody responders, suggesting a role for antibody in resistance to H.polygyrus infections in mice (Jenkins & Carrington,1981).

Sitepu & Dobson(1982) have selected high and low immune responder mice from a outbred Quackenbush stock. The two selected lines of mice differed in their antibody responses to H.polygyrus and the phenotypic character:

faecal parasite egg counts, but less so in their mean worm burdens. The Sitepu and Dobson's high and low responder lines of mice have also been found to have differential responses to parasitic infections like Salmonella typhymurium (Sitepu,Brindley & Dobson,1985) and Toxocara canis (Brindley et al,1985).

The size, duration and history of previous exposure to H.polygyrus have been observed to influence the outcome of infection in mice. As first described by Van Zandt(1961), removal of worms with drugs appears to have the effect of enhancing immunity. Additionally, sex and the genetic background of the host have also been found to be important in the generation of immunity in secondary infections.

Hosier & Feller(1973) observed that female outbred ICR mice were immune following primary infection with 200 H.polygyrus larvae and were refractory after two infections of 500 L3 each, interspersed with drug treatment. Panter(1969) reported increased resistance in outbred 'white mice' given 200 L3 twice and suggested that immunity was directed against the incoming larvae. Cypess et al (1973) also confirmed that outbred S-W female mice harboured fewer challenge worms than males when given a smaller dose of 50 L3 at three weekly intervals.

Male ICR mice were less well protected by single graded doses (10-500L3) of H.polygyrus than by small doses of 50 L3 given twice(Hosier,1974), compared to the protection level observed in female mice (Hosier & Feller,1973). Resistance in outbred CFW mice was greater than that observed in inbred C57BL/6 when these two mouse strains were infected with 10, 20 or 200 L3 H.polygyrus twice (Hepler et al,1976).

The designation of strains as 'high' and 'low' responders is subjective. The definition depends on what variables are measured (e.g. larval establishment, adult worm survival, mouse mortality etc.). Nevertheless, it is generally accepted that the C57BL/6, C57BL/10, C3H and CBA mouse strains are low responders to H.polygyrus (Prowse & Mitchell,1980; Cypess & Zidian,1975; Prowse et al, 1979; Hepler et al,1976; Behnke & Robinson,1985) while the SJL, NIH and LAF1 mouse strains are high responders Behnke & Robinson,1985; Jacobson et al,1982; Prowse & Mitchell,1980; Sayles & Jacobson,1983).

The significance of size of previous infection on the development of acquired resistance has been tested in the high responder NIH mouse strain. Between 90-99% protection from challenge infections was observed in NIH mice independent of whether the primary infection was small (10L3) or large (500L3) (Behnke & Robinson,1985).

In the Quackenbush mouse strain, a curvilinear relationship between the size of primary infection and number of worms recovered from a challenge infection has been described (Dobson, Sitepu & Brindley,1985). More worms were recovered from challenge infections following a high initial dose than lower doses, and it has been suggested that high-dose immunosuppression of the host was responsible for generating such a pattern (Dobson et al,1985). It was estimated that this apparent host immunosuppression by the parasite decayed over a period of 7 weeks after anthelmintic termination of the primary infection (Dobson et al,1985).

Enhanced resistance to H.polygyrus in mice has been reported when the immunizing regime employed small doses of larvae given repeatedly to mice at weekly intervals, interspersed with chemotherapy (Van Zandt,1961; Panter, 1969; Van Zandt et al,1973; Bartlett & Ball,1974; Behnke & Wakelin,1977; Kerboeuf & Jolivet,1980). These authors demonstrated that resistance to H.polygyrus in laboratory mice was some function of the size, duration and history of past exposure to infection and genetical background of the mouse.

The possibility that resistance in mice to H.polygyrus could be non-specific, rather than specific, exists, and has not been investigated satisfactorily (Wakelin,1978;1984).

Mucus trapping and exclusion of parasites has been implicated (Miller et al,1981). Evidence for increased goblet cell production in rats infected with Nippostrongylus brasiliensis has been presented (Uber et al,1980). Mastocytosis or increased mast cell production in N.brasiliensis (Miller & Jarret,1971; Nawa & Miller,1979; Befus & Bienenstock, 1979;1981) and Trichinella spiralis infections in rats (Alizadeh & Murell,1984; Ruitenberg & Elgersma,1976; Alizadeh & Wakelin,1982), has been observed. However, much uncertainty remains concerning the relevance of mastocytosis and goblet cell hyperplasia in the manifestation of rapid expulsion of gut parasites, since mice genetically deficient in mast cells (w/w^{v}) can reject worms (Uber et al,1980; Jarret et al,1980; Mitchell et al, 1983; Crowle & Reed,1981; Kojima et al,1980; Ha et al,1983).

The role of mast cells in immune expulsion of H.polygyrus by mice has yet to be shown. It remains to be demonstrated, for instance, that high responder mice readily produce more mast and goblet cells than do low responders. It has been shown that gastritis in mice exposed to multiple H.polygyrus infection is associated with increased numbers of eosinophils, neutrophils and mast cells in the lamina propria (Liu et al,1974).

Of particular interest is the observation that mice can be immunized by vaccination with living, dead or irradiated infective stages as well as by adult worms

or their 'excretory-secretory' products. Variables that influence the effective immunization of mice against H.polygyrus, include the route of vaccination viz. oral, subcutaneous(s.c.), intraperitoneal (i.p.) and intravenous (i.v.); the size and number of vaccination doses as well as host related factors like mouse age, sex and genetic background.

In general, the oral route of vaccination has been found to be superior to either the subcutaneous or intraperitoneal routes (Jones,1974; Jones & Rubin,1974a; Lueker & Hepler,1975; Hepler et al,1976; Chaicumpa et al. 1977). Once induced by this route, immunity appears to last for at least 30 weeks in Swiss mice (Rubin et al,1971). The age of mice at vaccination was found to be important, mice older than 11 weeks being more resistant than young mice (Rubin et al,1971). Protection afforded by subcutaneous vaccination of inbred C57BL/6J and Balb/c with 400 L3 was less than 30% (Rubin, et al,1971) and high protection levels could only be achieved by oral stimulation with as few as 200 L3 in inbred C57BL/6J, C3H/HeJ, BALB/cJ and AKR/J mouse strains (Lueker & Hepler,1975). Immunity following vaccination appears to be good in outbred CFW (Lueker et al,1968; Hepler et al, 1976) and ICR mice (Hosier & Feller,1973; Hosier et al,1974; Cypess & Zidian,1975) although contradictory results have been published by Chowaniec et al,(1972).

Swiss mice vaccinated i.v. with as few as 200 L3 H.polygyrus three times every 14 days were afforded significant protection as were mice vaccinated i.p. (Chaicumpa et al,1977). In the former case (i.v.), immunity persisted for at least 150 days. Protection failed to develop in mice immunized with freeze-thawed larvae (Chaicumpa et al,1977).

Oral immunization of female Balb/c mice with few living adult worms was more successful than using dead worms given either i.p. or by subcutaneous implantations (Hurley et al,1980). In vitro released (IVR) or excretory-secretory products were only effectively immunogenic at high doses in the presence of Freund's Complete Adjuvant (FCA) and divergent responses from the low responder CBA and high responder Balb/c mice were observed (Hurley et al, 1980). Vaccination of mice with soluble somatic protein or particulate antigen in FCA (Cypess,1970; Van Zandt,1971; Hurley et al,1980; Fernando-Monroy,Brindley & Dobson,1985) or a double emulsion adjuvant (Goven & DeBuysscher,1980) all gave low to moderate (30-50%) protection to H.polygyrus. Mitchell & Munoz(1983) have reported greater than 95% protection of the susceptible C57BL/6 mouse strain with i.p. injection of 20-30 adult H.polygyrus together with antigens from the bacterium, Bordotella pertussis. Considerable strain variation has been observed in mice vaccinated using this antigen plus adult worms (Mitchell & Cruise,1984).

The overall results are encouraging in so far as they point to the efficacy of a subset of antigens from the parasite as being funtionally host-protective even in strains of mice regarded as low responders. They augur well for the exploitation of parasite antigens in inducing immunity to intestinal nematodes.

Irradiated larval H.polygyrus can induce protection in mice (Behnke & Parish,1980; Hagan, Behnke & Parish,1981). Protection can also be induced in low responder C57BL/6 mice and high responder NIH mice. The protection appears to last for more than 10 weeks (Behnke & Robinson,1985). Attenuation by cobalt 60 irradiation affects the growth, survivorship and fecundity of the worms. The precise mechanism of immunity induced via infection of mice with irradiated larvae is not known (Behnke & Parish,1980).

On the basis of the knowledge that immunity induced by vaccination of mice is mainly directed against larval stages (Chaicumpa et al,1977; Hagan et al,1981), it has been suggested that the possible mechanism of induction of immunity is the destruction of encysted larvae by a process involving opsonizing antibody, assisting the antigenic recognition and eventual cell mediated killing of these larvae (Ninneman & Lueker,1974). Such host sensitization should, in theory, be able to convert low responders into high responders by by-passing the host

immunosuppression normally induced in certain strains of mice by larvae and adults of H.polygyrus and their products (Behnke et al,1983; Cayzer & Dobson,1983; Pritchard & Behnke,1985). It has been observed that steroids inhibit the expression of immunity in s.c. but not in orally vaccinated CFW mice. This observation has been interpreted as a demonstration of the efficient presentation of the antigen by macrophages during the oral route of vaccination (Hepler et al,1976).

Among the many requirements for a successful vaccine against infectious diseases are (i) the ability of the vaccine to induce a high degree of protection, (ii) to induce less immunopathology to the recipient than non-recipients and (iii) to confer long-lasting immunity (Ada,1982). With respect to H.polygyrus or hookworms, oral vaccination with live infective stages, although highly protective, suffers from the disadvantage that re-infections occur rapidly (Miller,1971;1978;1979).

Vaccination of outbred CFW mice with 50 normal L3 H.polygyrus induced resistance lasting for up to 50 weeks (Van Zandt et al,1973). Behnke & Robinson (1985) have found that high responder NIH mice could be protected for over 5 weeks. These results suggest that once induced, immunity to H.polygyrus in mice is of long duration.

The demonstration of acquired immunity to mouse and dog hookworms suggests that immunity to hookworm in man may be expressed and is protective (Carr & Pritchard, 1986; Carrol & Grove,1985; Ogilvie et al,1978; Ogilvie & da Sivigny,1982). Although we rely heavily on experimental analogues for the understanding of immunity in man, the available evidence warrants a re-appraisal of the manifestations of resistance to hookworm in man and increased research into the mechanisms modulating immunity if vaccines are to be seriously considered in the control of medically important helminths. In parallel to such developments, the acquisition of knowledge on how best to use these vaccines in a community is of high priority (Anderson & May,1985b,c).

1.5 Host-parasite population dynamics

Mathematical analyses of the population dynamics of the major helminth parasites of man have highlighted some general concepts essential for an understanding of the biological processes which operate in the regulation of parasite abundance (Anderson,1982b; Anderson & May,1978; May & Anderson,1978). Mathematical models are also helpful in the design of optimal community-based control programmes (Anderson & May,1982; Anderson & May,1985a,b,c).

The time scales on which host and parasite population changes operate, density-dependent regulatory processes, statistical distribution of parasite numbers per host,

reproductive biology and the transmission potential of the parasite, have all been identified as major factors controlling the transmission dynamics of helminth parasites (Anderson & May,1982).

Relative time scales depend on the average expected life span of the definitive host and the developmental stages of the parasite. In the case of H.polygyrus, the mouse has an average life span of about 12 weeks in its natural habitat (Flowerdew,1985) and between 1 and 1½ years in the laboratory (Bellamy,1981). The observed maximum life span of adult H.polygyrus is 8 months (Erhenford,1954), but the average life expectancy in the laboratory is 9-12 weeks (Keymer, 1985). Infective stages of H.polygyrus have an expected life span of about 12 weeks at 4°C (Kerboeuf,1978a). In nature, ambient conditions such as temperature, humidity, oxygen supply and natural predators and infectious diseases would be expected to reduce larval survivorship. Thus, under laboratory conditions, the dynamics of the adult parasite can operate on a similar scale to those of the mouse.

The regulation of host-parasite populations is manifested as non-linear density-dependent constraints on parasite establishment, growth, survivorship and fecundity in the absence of host immunological responses (Bradley,1972; Anderson,1978;1980; Keymer,1982). The net severity of these constraints on parasite populations

will be dictated by the statistical distribution of parasite numbers per host (Anderson & May,1982). Although these constraints are relatively easy to demonstrate in the laboratory, it remains to be seen whether they, in fact, play an important role in parasite population regulation in natural communities (Anderson,1982b).

Density-dependent per capita fecundity of H.polygyrus has been observed in outbred MF1 and CD1 mice (Kerboeuf, 1978b;1982; Keymer,1985). However, egg production per female H.polygyrus appears not to decline (with increasing parasite burden) below a certain threshold (Kerboeuf,1982; Kerboeuf & Jolivet,1984). Establishment of H.polygyrus during primary infections was found to be dependent on the infection dose within the range 10-300L3 (see Keymer, 1985). Survivorship of H.polygyrus is, however, thought to be independent of initial worm burden (Dobson,1985). It is largely determined by innate differences in mouse strains (Prowse et al,1979).

A common observation with most non-replicating helminth infections is that parasite distributions in their hosts are highly overdispersed or aggregated, with the majority of hosts harbouring a few or no worms at all (Crofton,1971; Bradley,1972; Anderson,1978). It has been suggested that one of the generative mechanisms of this observed parasite aggregation could be heterogeneity in exposure to infection as a consequence of aggregation in

the distribution of infective stages (Anderson & Gordon, 1982). Such mechanisms are likely to be important in the field (Anderson & Gordon,1982; Kennedy,1985) whereas mechanisms due to demographic and genetic factors e.g. via heterogeneity in immunological responsiveness, are more likely to be operative in controlled laboratory infections.

H.polygyrus was found to be highly aggregated in wild A.sylvaticus in Wales (Lewis,1968a) and it was thought that overdispersion in infective stages in the environment could be a determinant of aggregation of worms in the mice. It is difficult to ascribe a definite mechanism to this observed pattern in field studies as the variables are numerous. However, the demonstration in man of parasite aggregation especially when it is associated with predisposition to heavy (or light) infection, is of obvious significance to the control of human parasites by chemotherapy (Anderson & May,1982).

In dioecious nematode parasites like H.polygyrus, the probability of mating is critically dependent on the minimum average number of worms per host necessary for successful mating to occur (termed the breakpoint(MacDonald, 1965)), below which mating frequency is too low to maintain the parasite (Anderson & May,1982; Bradley & May, 1978). The level of the breakpoint will depend on the statistical distribution of parasite numbers per host and

and the sexual habits of the parasite (i.e. whether it is polygamous or monogamous). The breakpoint has been found to be as low as 0.3 hookworms/host in human communities (Anderson,1982a) and, as such, the concept of a breakpoint is thought to be of less practical significance to disease control than was initially suggested (Anderson,1980; Anderson & May,1982; Croll et al, 1982). Nothing is known about either the threshold probability of mating in the mouse/H.polygyrus system or the pattern of egg production in the field.

Another equally important factor in the transmission of parasites is the magnitude of their basic reproductive rate,Ro,(the average number of female offspring produced throughout the life time of a mature female parasite which in turn attain reproductive maturity in the absence of density-dependent constraints) (Anderson & May,1982). The value of this parameter must exceed unity (R_o>1) for parasite maintainance. Its value is typically in the region of 2-3 for hookworms (Anderson,1979;1980). A generalized target for helminth control programmes could, therefore, be to aim at reducing the value of R_o to below zero (Anderson,1982a). For geohelminths (e.g. Ascaris, hookworm, Trichuris, Toxocara) such a reduction in transmission potential implies control by a combination of improved sanitation, hygiene and education, and drug treatment (Anderson & May,1985a).

1.6 Human hookworm infection

Estimates of the prevalence of the major intestinal helminths of man throughout the world appear to have changed little in the last three decades since Stoll(1947) remarked upon the high abundance of these parasites (Anderson & May,1982; Warren,1981). Hookworm is especially prevalent world-wide in contrast to other important infections like schistosomiasis, filariasis, malaria, trypanosomiasis and leishmaniasis (W.H.O.,1981). Recent estimates suggest that over 600 million people are infected in the world today (Muller,1975; Peters, 1978; Schad & Banwell,1978; 1984; Warren,1981).

The two major species of human hookworm, Ancylostoma duodenale and Necator americanus occur sympatrically in many parts of Asia, Africa and America (Hoagland & Schad, 1978). In areas where hookworm is endemic, the prevalence of infection can be as high as 95% (Muller,1975), especially amongst teenagers (Anderson,1981; 1982b). Man is infected by ingestion and/or skin-penetration of the infective, non-feeding third-stage larva (L3). These larvae (L3) develop into sexually mature parasites in the small intestine, after a brief migration via the heart and lungs. Fertilized eggs are passed in the host faeces from 5-7 weeks after infection to the environment. They hatch to release, via several moults, the first (L1), second (L2) and the third (L3) larvae (Miller,1979).

1.6.1 Morbidity and pathophysiology

Hookworm infection is generally perceived as benign and inducing morbidity rather than mortality (Anderson,1980; Miller,1979). However, the long-term effects of persistent hookworm infection in man (sometimes acquired as early as 4-5 weeks after birth (Nwosu,1981)) remain relatively unexplored. Hookworms can induce primary and secondary anaemia in man through the loss of blood and iron reserves (Areckul,1975; Gilles *et al*,1964; Miller,1979; Roche & Layrisse,1966). The feeding activity of the worms with the use of the buccal plates and the aid of proteolytic enzymes (Hotez & Cerami,1983; Hotez *et al*,1985) causes leakage of plasma proteins, haemoglobin and loss of iron, folic acid and vitamin B12 (Roche & Layrisse,1966). Using ^{51}Cr-labelled red blood cell studies, blood loss due to *A.duodenale* was found to be 0.15 ml/day, five times higher than for *N.americanus* (Roche & Layrisse,1966). Malabsorption syndromes and mineral deficiencies (zinc and iron), acting synergistically with malnutrition and polyparasitism, will almost certainly adversely affect man (Bundy,1985; Stephenson *et al*,1985). Such effects underline the need to regard hookworm infection as a debilitating disease in need of medical intervention.

1.6.2 Epidemiology of hookworm infection

Major epidemiological studies whose conclusions are as relevant today as when the work was first undertaken were conducted in North America, Latin America and the Far East in the 1920's (Cort *et al*,1929; Docherty,1926; Hill,1926; Sweet,1925). Recently, analyses of quantitative epidemiological studies on hookworm have been described (Anderson,1980; 1981; 1982b; Anderson & May,1985a; Schad & Anderson,1985). Age-prevalence and -intensity profiles for hookworm in India (Chowdhury & Schiller,1968; Schad, Nawalinski & Kochar,1983); Taiwan (Hsieh,1970); Phillippines (Pesigan *et al*,1958); Sri Lanka (Fernando & Balasuriya,1976); Zambia (Wenlock,1978); Thailand (Papasurathorn *et al*,1967); Iran (Anderson,1982b; Croll & Ghadirian,1981) and the Carribean (Bundy,1985), show a similar pattern of an increase in prevalence and intensity during the early teens and stable plateau or decrease in intensity of infection (depending on the area) in the older age-classes of the population (Anderson & May,1985a). Mathematical models have suggested that the observed trends could be explained by postulating either an age-dependent force of infection or the acquisition of immunity (Anderson,1982b; Anderson & May,1985a).

Quantitative estimates of the rate parameters regulating the transmission success of hookworm may differ from one locality to another. In general, hookworm appears to have a basic reproductive rate, R_o, of around 2-3 and an

average adult worm life expectancy of about 2-4 years (Anderson,1982b). Together with the highly aggregated distribution of human hookworm (the negative binomial parameter, k, has been estimated at 0.3) it is not surprising that hookworm is a persistent infection (Anderson,1980). These observations imply that the parasite population is likely to bounce back to its equilibrium level following chemotherapeutic intervention (Anderson & May,1982). Recent epidemiological studies of intestinal helminth infections in humans in India suggest that the time it takes for hookworm infection (measured by worm counts) to reach 30% of its pre-control level is about 1 year (Elkins, Elkins & Anderson,1986).

Another biological feature of human hookworm infection relevant to its control is the recently described predisposition to hookworm infection in man (Schad & Anderson,1985). The factors that predispose individuals to hookworm are little understood, but, behavioural, genetic and nutritional factors are believed to be important (Schad & Anderson,1985). Community control of both morbidity and transmission of hookworm may benefit from optimal strategies involving selective or targetted treatment as opposed to repeated mass treatment (Anderson & Medley,1985; Anderson & May,1982; Warren,1981; Warren & Mahmoud,1976).

The mode of transmission of hookworm is believed to be via repeated low-level (=trickle) infections (Schad et al,1983) due to the overdispersed distribution of infective larvae in the environment (Schad et al,1983; Hominick, Dean & Schad,1986; personal comm. Udonsi et al,1981). Exposure to infection may be temporally discontinuous and mirror seasonal abundance of larvae (Schad et al,1983; Udonsi et al, 1983). Within each age-class of the human community, seasonally convex age-intensity curves with a general trend of increasing intensity with age may be common in Monsoon areas (see Schad et al,1983).

Studies in the laboratory involving repeated low-level infections in mouse populations, provide a means for examining the relevance of different factors (such as the intensity of exposure to infection) as determinants of observed changes in helminth abundance with host age.

1.7 Aims of the project

Despite the suggestion by Ball & Bartlett (1972) that H.polygyrus in outbred mice could be used as a laboratory model of endemic hookworm infection in man, very little experimental and quantitative ecological or epidemiological work has been done to bridge the gap in our present understanding of the epidemiology of intestinal nematode infections of man.

The features of human hookworm infection which are of relevance to the objectives of this project are that the infection is chronic; prevalence and intensity of infection are stable over a wide range of age-classes, the highest intensity of infection being observed in teenagers; and that a large percentage of the worms are harboured by only a small percentage of the host community (Anderson,1982b; Croll & Ghadirian,1981). Similar features are also characteristic of repeated H.polygyrus infections in outbred laboratory mice (Ball & Bartlett,1972) and in natural infections of wild rodents (section 1.3). An interesting question is, therefore, whether heterogeneity in host genetic background and rates of exposure to infection are linked with the generation of these patterns of infection. An ecological approach is employed involving the exposure of cohorts of mice to repeated infection at varying levels of infection in a similar manner to recent experimental studies of the population dynamics of Schistosoma mansoni in mice (Crombie & Anderson,1985; Anderson & Crombie,1985). This study specifically addresses the role of host genetics as a determinant of the dynamics of infection.

This dissertation is organised as follows. The materials and methods used in this study are detailed in chapter 2. In chapter 3, the heritability of the innate host response to H.polygyrus by the selection of 'high' and 'low' responder mice from a heterogeneous outbred mouse population is examined. The population biology of H.polygyrus in inbred and outbred mice is considered in

chapter 4 with the aim of obtaining estimates of the rate parameters controlling parasite abundance in laboratory mice. These estimate include rates of parasite establishment, survivorship and fecundity, and their dependence on the density and age of the parasite. The influence of previous experience of infection in outbred mice on the subsequent acquisition of resistance to varying challenge infections with H.polygyrus is also examined in chapter 4. In past studies, resistance to challenge infections is usually assessed using a single challenge dose.

The dynamics of repeated infections of low and high responder inbred mice (chosen so as to differ in their H-2 haplotypes) with varying levels of H.polygyrus are reported in chapter 5. Mathematical models of host-helminth populations developed by Anderson & May(1985b) are employed in the analysis of the experimental results. These models incorporate an assumption of reduced establishment of H.polygyrus in the two mouse strains as a function of the accumulated past experience of infection. The models are extended to include the increased parasite death rate under conditions of repeated infection.

The impact of chemotherapy in mice treated either early or late during trickle infection and their rate of re-infection are also examined. The relevance of vaccination of mice with irradiated larvae and the long-term duration of host resistance induced by both chemotherapy and vaccination is also explored in chapter 5.

Finally, in chapter 6, serum anti-H.polygyrus antibodies in mice exposed to repeated infection are examined. The possibility of a differential ability of serum from the high and low responder mice to immunoprecipitate radio-labelled surface antigens of H.polygyrus is analysed (by SDS-PAGE and autoradiography).

The thesis ends (chapter 7) by summarizing the main findings of the study and discussing the relevance and implications of the results to the transmission of intestinal nematode parasites of man. Future directions in research are also considered in chapter 7.

Chapter 2

Materials and Methods

2.1 Origin and maintainance of the laboratory mouse host

Outbred MF1 mice, originally purchased from Olac (Bicester,U.K.), were bred under conventional conditions initially at Queen Mary College and later at the Department of Pure and Applied Biology, Imperial College. All mice were kept in plastic (PVC) isolators (Vickers,UK and Isotech, Bicester,UK) fitted with air supply, humidity and temperature controls. Mice were housed in standard cages in groups of 5-10 animals per cage and fed on laboratory pellets (Oxoid 41B) and given water ad libitum. The bottom of cages were lined with wood chips and were changed at least once a week in order to minimize auto-infection with the nematodes Aspiculuris tetraptera, Syphacia obvelata and Heligmosomoides polygyrus.

Inbred CBA/Ca and NIH mice were purchased from Olac as required and additional CBA/Ca mice were obtained from stocks maintained in the animal unit at Imperial College. Mice were marked individually using an ear-punch marking system.

2.2 Origin and maintainance of the parasite

Heligmosomoides polygyrus (=Nematospiroides dubius) was originally obtained from Dr. John Lewis, Royal Holloway College, London. Two infected MF1 mice were used to build up the culture. Dr. Lewis's stock was obtained from the

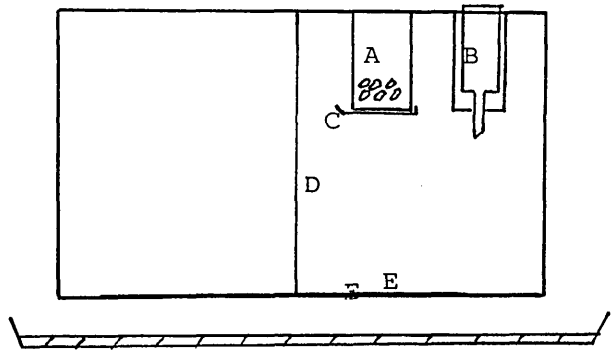
Wellcome Laboratories in Kent and passaged in outbred ASH, CD1 and MF1 mice consecutively. This parasite strain is believed to have been passaged in laboratory mice for several decades.

2.2.1 Culture of H.polygyrus

To obtain mouse faecal pellets containing worm eggs, passage mice were housed in groups of up to 10 in cages modified to enable faecal collection (Fig.2.1a). They had an open bottom, with a metal grid large enough to allow faecal pellets to pass through. A plastic tray covered with moist tissue was placed about 1.5-2.0 cms below the cage and mice were introduced into the cage with only water (no food) overnight.

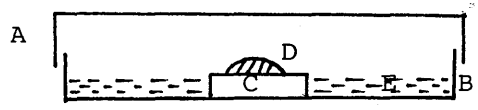
Next morning, the total faecal output was collected into clean beakers and worm eggs were extracted and cultured following a modification of the methods described by M.A.F.F.(1977) and Chaicumpa *et al*,(1977). Briefly, the moist faeces were gently crushed, using a spatula or pestle and mortar, into a thick suspension in distilled water. Large sedimentation jars were fitted with nylon, mesh size 250 μm, and the faecal suspension was generously washed through with distilled water. The eggs were allowed to sediment to the bottom of the jar for 1-2h. Meanwhile, culture plates were prepared as follows.

(a)



A- food basket
B- water bottles
C- metal collector
D- perspex divider
E- metal grid
F- plastic tray with layer of wet tissue

(b)



A- large petri-dish cover
B- large petri-dish
C- small petri-dish
D- faecal smear
E- distilled water

Figure 2.1. Apparatus for the collection of, and culture culture of H.polygyrus from mouse faeces in the laboratory.

Small glass petri-dishes, 4.5 cm diameter, were placed upside down inside large petri-dishes, 14.0 cm diameter, with lids. Strips of No.1 Whatman qualitative filter paper, 3-4 strips thick, were cut into rectangular pieces 3cm wide by 11cm long. These were placed over the small petri-dish such that the ends went underneath the petri-dish (Fig.2.1b). In this way, the filter paper would absorb water in the large petri-dish and remain moist.

At the end of the sedimentation period the supernatant was discarded and the sediment washed into 15 ml plastic centrifuge tubes. The faecal specimen was centrifuged for 5 mins at 3,500-4,000 R.P.M. and the supernatant discarded. The process was repeated again in order to wash the faecal pellet thoroughly. After the final wash, the supernatant was discarded and the faecal pellet was scooped (using a clean spatula) onto the moistened filter paper inside the culture dish and spread evenly over the surface. Finally, a layer of distilled water was poured into the large petri-dish to a level below the maximum height of the smaller petri-dish. The petri-dish assembly was then cultured at 25±1°C in an incubator for 6-7 days. Occas ionally, the lid was opened for about 5-10 mins to permit aeration of the culture.

Hatched larvae migrate away from the faecal smudge into the surrounding water or wait upright on the edge of the filter paper. Six to seven days later, infective larvae were harvested by pipetting all the water into 50ml sterilin tubes (v-bottom) and the edge of the filter paper was washed carefully to remove adherent larvae. The larvae were allowed to settle to the bottom of the tube and the supernatant was pipetted out. The parasites were washed several times with distilled water to remove debris and bacteria before storage in a shallow layer of water in sealed bottles at 4°C.

2.3 Infection of the laboratory mouse

Mice were infected individually by stomach intubation. A known aliquot of parasites, estimated by counting larvae under a dissection microscope in 0.1 ml samples, was sucked up in a 1 ml plastic syring fitted with a 19-gauge needle (Sterilin), modified by fitting a blunt tip and plastic bob to facilitate swallowing by the mouse and to prevent injury to the host. Mice were held in the left hand with the thumb and fore-finger holding onto the scruff of the neck to prevent head movement (and bit/ing!) with the tail and body of mouse inside the other three fingers. A 100µl volume of the requisite number of larvae (e.g. 50L3/0.1ml) was given to the mouse and care was taken to ensure that the suspension did not accidentally pass into the trachea. Infected mice were then transferred to clean cages.

2.4 Faecal egg collection and counting techniques

In all experiments, faeces were collected from individual mice for the enumeration of parasite egg counts based on both weight (eggs per gram) and unit time (eggs per unit time) basis. Faecal collection cages, described in section 2.2, were modified to accommodate two mice per cage by inserting a transparent perspex partition which divided the cage into two living arenas. The top metal grid was replaced by a perspex top with two openings on either side to accommodate the food and water baskets. A metal collector was fitted onto the food basket in order to prevent food particles from contaminating the faeces at the bottom of the cage.

Faeces were collected from individual mice, initially for 24h but later, for 16h (overnight) since it was found that mice produced an equivalent amount of faeces during both periods, with little change in eggs per gram (epg) or eggs per 16h. Also, in long-term experiments, heavily infected mice were too weak to stay in cages for 24h since in most instances, they neither fed nor drank water during these isolation periods. In addition, some of the eggs from 24h faecal collections already contained mobile larvae. Some eggs appeared to be in the process of hatching; this suggested that egg count errors due to hatching were likely to occur in 24h collections.

Faecal collections over 16h were therefore obtained on wet tissue as in section 2.2. Faecal pellets were collected individually, using fine forceps, and placed in marked, 10 ml sterilin tubes with lids of known weight. Faeces were always processed within 24h after maintenance at 4°C. Control experiments revealed that total egg counts in faeces kept at 4° C tended to decline to approximately half the initial count over a period of one week (presumably as a consequence of the degradation of dead eggs). Fecundity was expressed as eggs per 16h period.

2.4.1 Modified McMaster egg counting technique

Parasite eggs were enumerated by a modification of the McMaster technique (MAFF,1977) which took account of the fact that mouse faeces often weighed less than one gram. The total faecal output over 16h of known weight from each mouse was ground into a thick suspension inside the collection tube with a flotation fluid consisting of saturated magnesium sulphate with specific gravity of 1.25. The suspension was then poured through muslin, mesh size 150-200μ, to remove bulky debris and then rinsed carefully with a known volume of up to 30ml magnesium sulphate per gram of faeces. Care was taken to ensure that no eggs were lost. This procedure was preferred to washing the suspension into sedimentation jars with distilled water because it by-passed several steps like sedimentation (½ - 2h) and centrifugation. Losses due to the transfer

of the suspension into tubes were also minimized. To process one specimen took approximately 3-5 mins; up to 30 specimen could be processed in one day as opposed to only 10 by the original method (MAFF,1977).

A known volume of the suspension was drawn off with a pasteur pipette and placed into a McMaster slide where the eggs were allowed to float to the surface. All the eggs present under the grid were then counted using a light binocular microscope at low power (x40). From 4-6 readings per sample were made and the eggs per 16h were calculated on the assumption that the volume under the grid was 0.15 ml. To obtain eggs per gram, this number (eggs/16h) was divided by the total weight of the stool specimen.

2.5 Post-mortem dissection of mice

Mice were killed under either diethyl ether or chloroform anaesthesia by cervical dislocation. They were flayed, the peritoneum opened and the intestines stripped from the mesenteries, starting from the pyloric sphincter and moving down the length of the small intestine until the ileo-caecal valve. The whole intestine was excised, split open and placed into a clean petri-dish containing 0.85% saline. Due to a high degree of fibrous tissue and/or cysts developing during trickle infections, care was necessary in excising the intestine lest it was broken.

The petri-dish was incubated for 1-2h at 37°C to allow the worms to migrate away from the intestinal tissue. All worms were then sexed and counted individually or in pairs. Worms were fixed in 10% formalin and stored in sealed sterilin tubes for measurement of worm length.

2.5.1 Larval worm recovery

Encysted fourth-stage larvae could be counted before opening up the gut by using the technique of Chaicumpa et al (1977). Briefly, the wole gut was placed between two perspex slides 30 cm x 2 cm, and, by pressing the intestine sufficiently, the larvae and cysts could be seen and counted under the dissecting microscope. Alternatively, when all larvae failed to migrate away from the tissue at 37°C for 1-2h, a digestion technique was used (McNeilson et al,1973). The intestine, after counting and removing all adult worms and some larvae, was placed in a clean petri-dish containing the digestion fluid (Herlich's) consisting of pepsin plus HCl, overnight at 37°C. The following morning, all larvae were counted under the dissecting microscope.

2.5.2 Recovery of fourth-stage larvae

To obtain fourth-stage larvae(L4) as a source of anti gen, infected mice were killed as before (section 2.5) and the intestine, freed from the mesenteries, was treated

as follows. The intestine was either tied with cotton threads at both ends to avoid spillage of the contents and then incubated in saline at 37°C for up to 4h (Ey, Prowse and Jenkin,1981) or split open and incubated in normal saline or phosphate buffered saline at 37°C for 2h. Larvae embedded in the externo-muscularis mucosa migrate out into the medium. These larvae can then be picked up individually, washed extensively before use (see section 2.8.1) or stored at -20°C.

2.6 Drug treatment (chemotherapy)

The original stock of MF1 outbred mice used for the breeding experiment (chapter 3) were treated to remove pinworm infection with viprynium sulphate (Parke-Davis, UK) at 50 mg/kg body weight. Pyrantel tartrate (Pfizer, UK) was used to clear worms from infected mice. It is effective chiefly against adult worms, when applied at 50 mg/kg body weight (Behnke & Wakelin,1977). When both adult and larval worms were the target of drug treatment, ivermectin (Merck & Sharpe,UK) diluted in the solvent propylene glycol (propane 1-,2-diol)(BDH) was given to mice at the rate of 10 mg/kg body weight (Sayles & Jacobson,1983). All drugs were administered by stomach intubation in a fresh preparation formulated to give 100 µl of drug for 20g body weight of mouse. Care was taken to provide a drug-treatment control group whenever mice were dosed with these drugs. This was done to check the efficacy of the drugs under our experimental conditions.

2.7 Humoral antibody responses

2.7.1 Serum collection

In some selected experiments, serum was obtained from individual mice by cardiac puncture. Mice were anaesthetized with chloroform, killed by cervical dislocation and quickly flayed. The thoracic cavity was opened to expose the heart and a 19 gauge sterilin needle fitted to a 1 ml sterilin syringe was used to suck up about 0.5 ml blood. Spilled blood and also blood clots were collected into a 1.5 ml Eppendorf. Individual sera were allowed to clot at room temperature for 1h and then left overnight at 4°C. The specimen was centrifuged at 5,000 RPM at 4°C for 10 mins and the serum was pipetted out using a clean Gilson pipette-man tip into another 1.5 ml Eppendorf. The serum was kept at -20°C until use.

2.7.2 Antigen preparation

Ten to twenty male outbred MF1 mice were each infected with 500 H.polygyrus larvae in order to raise large amounts of antigen. Antigen was prepared from 6d old fourth stage larvae (D6L4) and adult worms (AH) obtained as described in section 2.5. After extensive washing of both larvae and adults in normal saline, they were ground in 1 ml Tris-buffer containing protease inhibitors in a

glass-glass tissue homogenizer (Jencons). The suspension was then centrifuged for 30 mins at 10,000 RPM at 4°C (Sorvall centrifuge). The supernatant, AH or D6L4 homogenate, was dispensed into 1 ml Eppendorfs in 100µl volumes and stored at -20°C until used. Protease inhibitors included 25µg/ml N-α-p-tosyl-L-lysine-chloro-methyl ketone (TLCK); 25µg/ml L-1-tosylamide-2-phenyl ethyl-chloroethylketone (TPCK); 1mM phenylmethyl sulphonyl fluoride (PMSF); 5µM pepstatin and 1mM ethylene diamine tetra acetic acid (EDTA). These were all supplied by Sigma.

2.7.3 Protein determination

The concentration of protein in the antigen homogenates was determined by the Bradford method (Bradford,1976; Sedmark & Grossberg,1977) using the commercially prepared Bio-Rad kit. Bovine serum albumin (BSA) was used to construct a standard curve. Adult homogenate was adjusted to a stock concentration of 5mg/ml.

2.7.4 Enzyme-linked immunosorbent assay (ELISA)

Anti-*H.polygyrus* antibodies in mouse sera were measured using a modification of an enzyme-linked immuno-sorbent assay (Voller, Bartlett & Bidwell,1976;1980; Engvall & Perlmann,1972; Pritchard, Behnke & Williams,1984).

Microtitre plates (Nunc 96F,Denmark or Dynatech,UK) were coated with 5µg/ml of AH prepared in carbonate-bicarbonate buffer, pH 9.6, by incubation of plates for 1h at 37°C and then overnight at 4°C. Preliminary chequer-board titration tests had shown that a 1:50 serum dilution was optimal for this optimal antigen concentration. Hence, all test sera were diluted 1:50 in wash buffer (0.1M PBS plus 0.5% Tween 20 (polyoxy-ethylene sorbitol monolaurate, Sigma)) containing 10% BSA to block any non-specific binding to the parasite antigen.

An automated plate washer (Titretek) was used for washing at 3min intervals in-between washes and all incubation steps were for 1h at 37°C. Except where mentioned, 200µl volumes were used throughout. IgM and IgG1 antibodies were measured using optimally diluted (1:1500) peroxidase-conjugated affinity purified rabbit anti-mouse IgM and IgG1 antisera (Miles,UK). Fresh substrate was made up with 10% ortho-phenylene diamine (OPD)(10mg/ml) in citrate-acetate buffer, pH 5.0, plus 0.001% 30% hydrogen peroxide (BDH). The plate was covered with aluminium foil and allowed to stand at room temperature for 30 mins. The reaction was stopped by the addition of 25µl 4M H_2SO_4 per well. The optical densities were read at 490 nm using an automated plate reader (Dynatech).

2.8 Characterization of antigens of H.polygyrus

2.8.1 Radio-labelling of parasites

Freshly acquired larval and adult H.polygyrus were washed extensively in PBS buffer prior to surface labelling with $Na^{125}I$ (Amersham,UK) by the Iodogen (1,3,4,6-tetrachloro-3α,6α-diphenylglycouril) method (Markwell & Fox, 1978; Fraker & Speck,1978). The parasites were transferred to 1.5 ml Eppendorf vials coated with 500ug dried Iodogen (Pierce Chem, Co.) and excess buffer used in transfer was removed. In a well vented fume cupboard, 500 µCi $Na^{125}I$ (=2.5µl) was then added to the vial and the labelling mixture agitated continuously at room temperature for 10 mins. A 10% v/v saturated tyrosine solution was then added to remove unreacted iodine, before the parasites were transferred to a 10 ml centrifuge tube and washed three times in cold 10 mM Tris buffer, pH 8.3, containing protease inhibitors.

2.8.2 Solubilization of surface antigens

Radio-labelled parasites were taken up in 1% sodium deoxycholate (NaDOC) solution in 10 mM Tris plus protease inhibitors for solubilization of surface antigens (Maizels et al,1986) and homogenized in the same buffer-detergent. The homogenates were centrifuged twice for 30 mins at 4°C, 10,000 xg. Supernatants were recovered as labelled surface antigen.

2.8.3 TCA precipitability

Supernates were assessed for labelling efficiency by precipitation with 10% trichloroacetic acid (TCA). 3 µl of normal rat serum was added to 3 µl of surface-labelled sample in a LP3 tube, vortexed, and the radioactivity was counted using a gamma counter. Tubes were then filled slowly with cold 10% TCA, while vorted mixing, and allowed to stand on ice for 1h. They were centrifuged twice for 10 mins at 4°C using a top-speed bench centrifuge. The supernatant was discarded and the pellet counted for radioactivity. This pellet was used for immunoprecipitation as described in the following section.

2.8.4 Immunoprecipitation and SDS-PAGE

Immunoprecipitates were manufactured by reacting a volume equivalent to $1x10^4$ c.p.m.(AH) and $1x10^5$(d6L4) in 50 µl PBS with 5µl test mouse serum in Eppendorfs and incubating overnight at 4°C. Antigen-antibody complexed were precipitated by the addition of 50 µl of a 10% suspension of Staphylococcus aureus (washed free of the formalin fixative) plus 5µl goat anti-mouse Ig. After incubation for 1h at 4°C, the precipitates were washed 3x in 10mM Tris buffer. The pellet was vortexed vigorously and re-solubilized in 40 µl of SDS-PAGE loading buffer containing 5% freshly added 2-mercaptoethanol (a reducing agent) and boiled at 100°C for 5 mins.

The iodinated immunoprecipitates were counted for radioactivity before loading onto gels for sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE). Homogeneous 10% polyacrilamide running gels and 5% stacking gels were use (Sutanto et al,1985). Iodinated molecular weight markers (Pharmacia), range 14.4-94 kda, were also included. Following fixation and vacuum drying, gels were taken for autoradiography by exposure to sensitized Fuji X-ray film using Ilford fast tungstate intensifying screens at -80°C for 10 days (Sutanto et al,1985).

2.9 Data analysis

The experimental data was analysed for significance by performing standard statistical tests (Sokal & Rolf,1982; Zar,1985) and statistical packages. Significance was accepted at the 95% level.

Chapter 3

Selection of high and low responders from outbred MF1 mice

3.1 Introduction

Recently, it has been increasingly accepted that populations of parasites within hosts are under some direct or indirect (e.g. via the immune system) genetic control by their hosts (Wakelin,1978b;1985a,b; Skamene et al,1980; Luzatto,1986). Most of the evidence is obtained from laboratory experimentation. This is due to the relative ease with which experimental host-parasite systems can be manipulated, the short generation times of the parasite and the ability to breed genetically defined strains of laboratory rodent hosts (Falconer,1981; Festing,1982; Hohenboken,1985).

It has been shown, for example, that mice can be selected for high and low responsiveness to the nematode parasites, Trichuris muris (Wakelin,1975), Trichinella spiralis (Wakelin,1980; Wassom,David & Gleich,1979; Wassom, Wakelin, Brooks, Krco & David,1984) and H.polygyrus (Brindley & Dobson,1981; Sitepu & Dobson,1982; Brindley, He, Sitepu, Pattie & Dobson,1986). Dineen & Wandon (1980) have reported a separation of high and low responder sheep to vaccination against Trichostrongylus colubriformis infection. Genetic control of other parasites such as the cestode Hymenolepis citelli (Wassom, DeWitt & Grundman, 1974; Arnason, Dick & Wassom,1986); the trematode, Schistosoma mansoni (Jones & K usel,1985) and the Leishmania protozoa (Blackwell,1985; Skamene, Gros, Forget, Kongshavn, St Charles & Taylor,1982) and particulate antigens (Biozzi et al,1979) have been described.

The association between the major histocompatibility complex in humans (HLA) and disease is also well known (Svejgaard,1980). The list is, however, largely made up of immunopathological diseases such as rheumatoid arthritis, dermatitis, coeliac, endocrine (e.g. diabetes) and neurological (e.g. multiple sclerosis) diseases plus a few viral and bacterial infections e.g. leprosy, herpes and tuberculosis (see Svejgaard,1980).

Genetic control of parasitic infections by their hosts can be expressed in several ways. Non-specific, innate resistant mechanisms usually form the first-line of defence against parasites (Wakelin,1985a). The rate of parasite establishment and/or expulsion are affected and may be under the control of several genes (=polygenic) - including non-H-2 linked genes, or a dominant autosomal gene as in H.citelli (Wassom et al,1974) and T.muris (Wakelin,1975).

Control of infection or disease by the host can also be manifest at the level of specific, passive and/or adaptive immunity. The genes involved may or may not be linked to the major histocompatibility complex (Wakelin, 1978b). Host genetic factors have been demonstrated in relation to the production of antibody- or cell-mediated immunity following exposure to infection or relevant antigens (Mitchell,1979). However, these effects are not always correlated with ecologically significant parameters such as worm burden and reproductive rate (see Wakelin,1985b) or disease (Jones & Kusel,1985; Blackwell,1985).

Inheritance of innate resistance and/or susceptibility to H.polygyrus has been shown by selection of high and low responder mice from an initial (Quackenbush x wild)F1 mouse population (Brindley & Dobson,1981). Presumably, the introduction of 'wild' type genes increased the genetic variability of the outbred Quackenbush mice. Partial linkage between the genes controlling innate and acquired immunity has been shown using challenge infections (Sitepu & Dobson,1982; Brindley & Dobson,1983).

The host-parasite system that was chosen to demonstrate the heritability of innate resistance/susceptibility to a nematode infection was outbred, laboratory MF1 and H.polygyrus. Following a standard dose of infective larvae, mice were designated as high and low responders to H.polygyrus on the basis of the phenotypic character 'total faecal parasite eggs/mouse/16h'. Mice excreting large numbers of parasite eggs/16h were considered low responders i.e. they harboured more worms than the high responders (those mice excreting few parasite eggs/16h). The shortcomings of the use of egg counts to predict parasite burdens will be discussed in this chapter and also in chapter 4.

Four generations of mice in these two 'lines' were selected in a two-way selection program (Falconer,1981). Environmental effects were kept to a minimum and controlled by maintaining an unselected outbred mouse population.

The long-term aim was, therefore, having demonstrated the genetic control of infection levels, to establish high and low responder mice. These selected mice could then be used to investigate the dynamics of parasite acquisition and loss under conditions of repeated exposure of mice to infection. The results of repeated infections in two inbred mouse strains designated as high and low responders to H.polygyrus will be reported in chapter 5. These two inbred mouse strains were chosen such that they were of different haplotypes (the major histocompatibility complex corresponding to HLA in man).

This chapter is organised as follows. First, the relevant theoretical background necessary for the analysis of selection experiments is reviewed in section 3.2, followed by a detailed account of the experimental design in section 3.3. An account of the results on the measurement of metric characters and estimates of their heritability over several generations is given in section 3.4. Finally, the results are discussed in relation to host-parasite population dynamics in section 3.5.

3.2 The theoretical model of selection

Before a discussion of the mathematical basis for the selection experiment, the statistical methods commonly used to analyse the resemblance between parent and offspring (in the absence of selection) will be briefly discussed. The theoretical details have been developed and discussed by Hill(1971;1972a,b), Falconer(1981) and Bulmer(1980). The purpose of this discussion is, therefore, to provide a template for the analysis of the data from the selection experiment.

In a randomly breeding population where there is neither interaction nor correlation between the environmental (V_E) and genetic (V_G) components of variance, the phenotypic variance (V_P) will be given by (Falconer,1981):

$$V_P = V_G + V_E \qquad [3.1]$$

where the environmental component of variance is the sum total of all variation due to, in this specific case, the technique of infection, inherent biological variability in parasite reproduction - including density-dependent effects on parasite fecundity and the environmental circumstances under which the experiment is conducted. If this environmental variance is kept to a minimum by careful experimental design, then the degree of genetic determination will be given by

$$V_G/V_P \qquad [3.2]$$

We can therefore define a further component, the additive variance, V_A, which specifies the degree of genetic resemblance between offspring and parents. The proportion of the additive variance over the phenotypic variance is defined as the estimate of the heritability, h^2, where

$$h^2 = V_A / V_P \qquad [3.3]$$

Thus, if the genetic character was measured between both parents and offspring, the regression coefficient (b_{AP}) is an estimate of the heritability

$$h^2 = b_{AP} \qquad [3.4]$$

In the case of offspring-one parent regression, the heritability estimate is given by

$$h^2 = 2b_{AP} \qquad [3.5]$$

This simple statistical procedure enables the estimation of the heritability of a character and its standard error in a randomly mating population. The underlying assumptions in the model are the linearity between the parental and offspring values and equality of variance (Hill,1971; Bulmer,1980).

If we denote the phenotypic value of one offspring per pair of parents at generation i as y_i and that of parents as x_i, then the slope, β, of the regression line (Bulmer,1980)

$$y_i = a + \beta x_i + e_i \qquad [3.6]$$

is estimated in the usual way by

$$\hat{\beta} = \sum (x_i - \bar{x})(y_i - \bar{y}) \; / \sum (x_i - \bar{x})^2 \qquad [3.7]$$

where $$\hat{\beta} = h^2 \qquad [3.8]$$

The residual mean square estimates the error term e_i

$$\text{var } (e_i) = (1 - \tfrac{1}{2}\beta^2)V \qquad [3.9]$$

Falconer(1981) estimates the variance associated with β in the case of offspring-mid/parent regression as

$$\text{var } (\beta) = \frac{1}{N-2}\left(\frac{\text{var } (x_i)}{\text{var } (y_i)} - \beta^2 \right) \qquad [3.10]$$

where N = number of families or paired observations, and var (x_i) and var (y_i) are the variances of the mean value of mid-parents and offspring, respectively. For offspring-one parent regression, the standard error of the estimated heritability is approximately

$$S.E.(h^2) = 2\,\sigma_\beta \qquad [3.11]$$

whereas for offspring-mid/parent regression, it is simply

$$S.E.(h^2) = \sigma_\beta \qquad [3.12]$$

So far, only empirical methods for estimating genetic parameters in the absence of selection have been considered. A linear model [3.6] was formulated to capture the essential features of offspring-parent regression with the additional assumptions of no epistasis i.e. no genetic and environmental interactions, and linkage disequilibrium.

Under selection, a different approach is needed. Selection has three main effects. First, the gene frequencies are likely to be altered independently of random drift and secondly, the assumption of equal variance is violated and hence, the regression may also change during selection. Thirdly, and clearly, during selection mating is assortative rather than random (Hill,1971;1972; Bulmer,1980; Falconer,1981).

Bulmer's(1980) empirical model assumes linear regression where the expectation of regression of offspring on parents is given by

$$E(Y_O | Y_P) = a + bY_P \qquad [3.13]$$

and Y_O and Y_P are the mean phenotypic values of offspring and parents respectively, assuming no sex differences. Following selection, the mean phenotypic value of k number of parents selected for breeding from N, the total population, will be $\bar{\bar{Y}}_P$. From equation [3.13], the expected phenotypic value in their offspring will be

$$E(Y_O | \bar{\bar{Y}}_P) = a + b\bar{\bar{Y}}_P \qquad [3.14]$$

and the predicted value, in the absence of selection, is from [3.6],

$$E'(Y_O | \bar{\bar{Y}}_P) = a + b\bar{\bar{Y}}'_P \qquad [3.15]$$

Therefore, the change in the mean value among parents due to selection or the selection differential,S, is given by

$$S = \bar{Y}_P - \bar{Y}'_P \qquad [3.16]$$

and the predicted response to selection (R) is the deviation of the mean phenotypic value of the offspring of selected parents from the whole parental generation before selection. Combination of equations [3.14] and [3.15] gives

$$R = b(\bar{Y}_P - \bar{Y}'_P) = bS \qquad [3.17]$$

which is equivalent to

$$R = \beta S = h^2 S \qquad [3.18]$$

and β is the regression coefficient and is defined, in the case of no sex differences) as $\beta = 2b$; and h^2 is the realized heritability. The realized heritability,h^2, can be of some predictive, practical value in breeding programmes (Falconer,1981). From the relationship

$$S = i\sigma_P \qquad [3.19]$$

it follows that the expected or predicted response, R, is

$$R = ih^2\sigma_P \qquad [3.20]$$

where σ_P = phenotypic standard deviation; i = intensity of selection.

The precision of the parameter, h^2, will be underestimated since the regression is computed from mean values only (Falconer,1981). Hill(1971) has discussed the optimal design to reduce this lack of precision. Divergent selection i.e. selection in two directions, and to a lesser extent, maintenance of a control, unselected population to monitor the effects of random drift improves the estimate (Hill,1971). Selection of parents reduces the variance in equation [3.10] and so will inbreeding (Falconer,1981). There is an optimal proportion of parents to be selected in order to reduce the error of the estimate and this proportion is a function of the heritability value (Hill,1971). In general, proportions of 0.15-0.20 are efficient (Soller & Genizi,1967; Hill, 1971). Replicated selection itself enables the calculation of the standard error, but may be excluded if it is very time consuming (Falconer,1981).

3.3 Experimental design

Fifty male and 50 female outbred MF1 parental stock (G.I) bred at Q.E.II College (see Materials and Methods) were brought into the Department of Pure & Applied Biology, Imperial College. All mice were individually marked and used at 6-8 weeks old. Information on parents, day of mating and weaning was kept. Weaned mice were housed in groups of 10 of each sex. Each mouse was infected with 100L3 H.polygyrus in 0.1ml distilled water after carefully

adjusting the concentration of larvae. Three weeks after infection, all mice were transferred to individual cages and the 16h faecal output of each mouse collected. Faecal parasite egg counts were performed from each mouse and expressed as eggs/16h. For practical purposes, the three breeding lines were infected at weekly intervals in order to facilitate egg counting.

Ten pairs of mice with the lowest egg counts (=high responders(H)) and 10 pairs of mice with the highest egg counts (=low responders(L)) were selected for paired mating (representing 20-30% of all mice). All other mice were killed and their worm burdens assessed. Mice selected for breeding were each treated with 50 mg/kg body weight pyrantel tartrate to remove all worms. In this way, the possibility of maternal transfer of protection was eliminated (Brindley & Dobson,1981). A control random (Rd) group of uninfected outbred MF1 mice was also maintained by breeding at least 10 pairs or in harems of 1 male to 5 females. Only, and all, the first litters were used for breeding in the next generation. Inbreeding was kept to a minimum by avoiding brother-sister matings where possible. In some cases, reproductive failure within the 3-6 weeks mating period occurred and such breeding pairs were excluded. Offspring from the three breeding lines were weaned from their mothers at 3 weeks of age and the sexes separated.

The progeny (G.II) of G.I mice were all infected and ranked in the same way as G.I for high and low faecal parasite egg counts. Mice from the Rd line were also infected to check the infectivity of larvae and acted as a 'breeding control' for environmental effects. This procedure was repeated until G.IV offspring were obtained.

3.4 Results from the selection experiment

3.4.1 The relationship between the total number of eggs/16h and the number of female H.polygyrus.

There was a significant positive, linear relationship between the number of female H.polygyrus and their 16h egg output from both male ($t=10.84$; D.F.=27; $P<0.001$) and female ($t=12.93$; D.F.=25; $P<0.001$) MF1 mice (Fig. 3.1a,b). The regressions of log(10) transformed egg counts (to normalize the data) on worm burden were also highly significant ($P<0.001$).

The results in Fig. 3.2 show the 95% confidence limits of the mean egg counts from groups of female worms (recovered from female MF1 in Rd G.I and G.II). They clearly indicate that (by lack of overlap between the 95% C.L. of the extreme groups) within the experimental range of infection dose (100 H.polygyrus) employed in this study, mice excreting many eggs, can be distinguished from those excreting few parasite eggs.

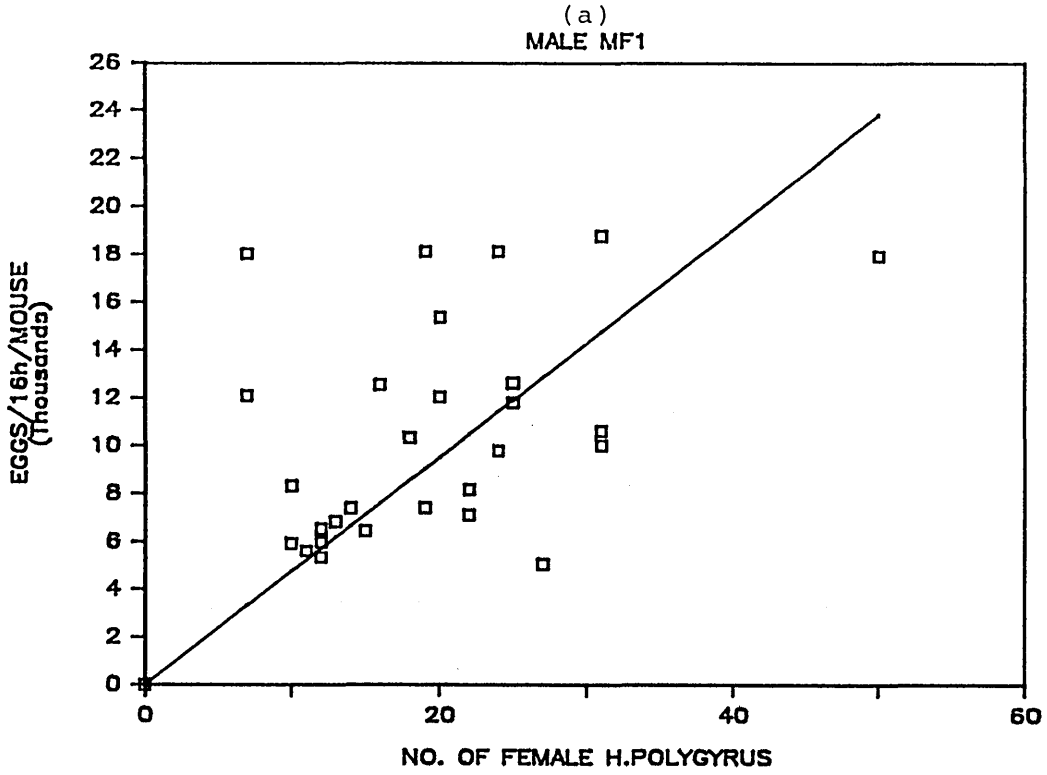
Figure 3.1. The relationship between the number of female H.polygyrus and the total number of eggs/16h/mouse from singly infected male (a) and female (b) outbred MF1 mice.

Squares - observed points; solid line - predictions of a best fit linear model (constrained through zero) of the form $y = bx$, with parameter values:

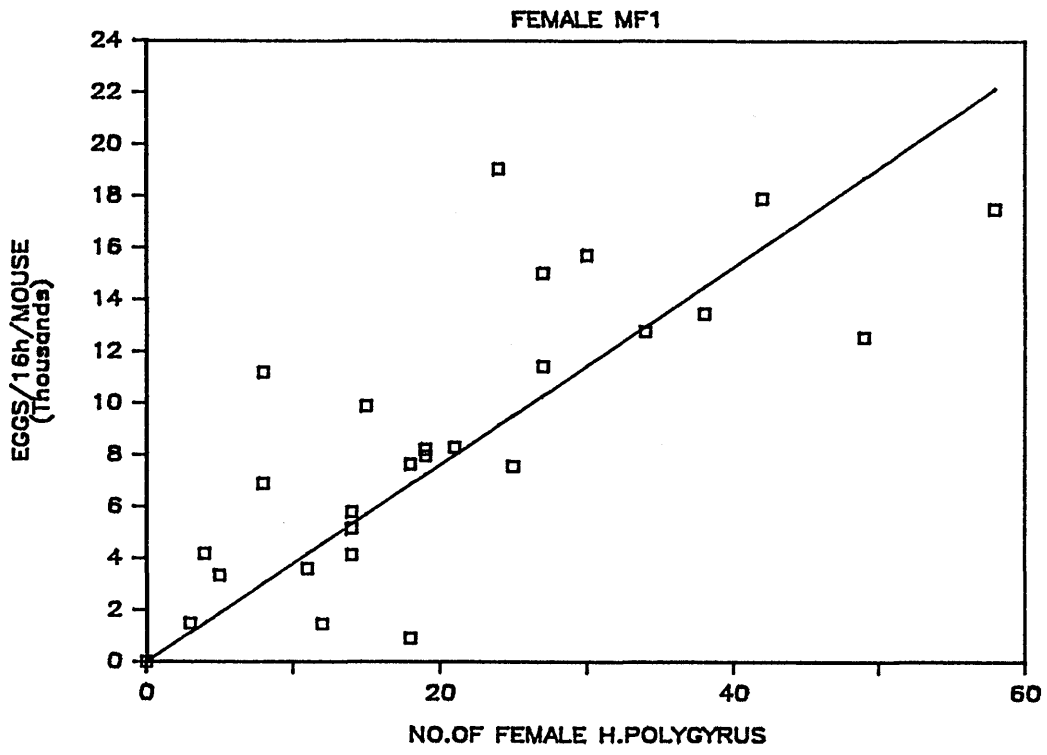
(a) $b = 477$; $P<0.001$;

(b) $b = 382$; $P<0.001$.

(a)


MALE MF1
EGGS/16h/MOUSE (Thousands)
NO. OF FEMALE H.POLYGYRUS
0
2
4
6
8
10
12
14
16
18
20
22
24
26
0
20
40
60

(b)


FEMALE MF1
EGGS/16h/MOUSE (Thousands)
NO.OF FEMALE H.POLYGYRUS
0
2
4
6
8
10
12
14
16
18
20
22
24
0
20
40
60

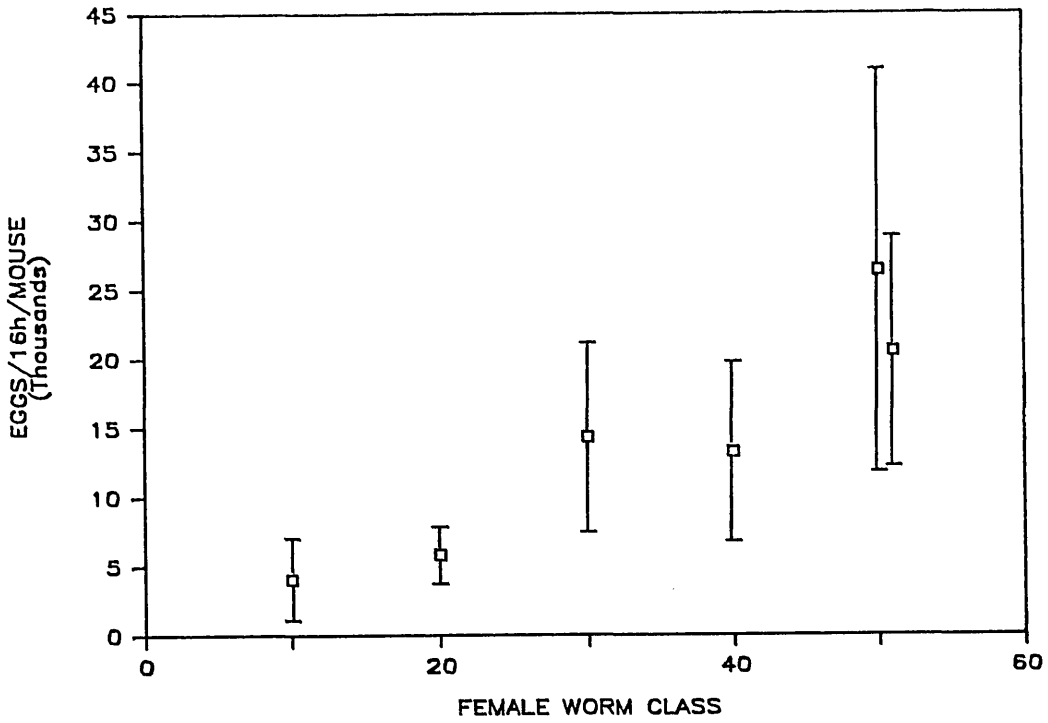


Figure 3.2. The relationship between the number of eggs per 16h per mouse and groups of female H.polygyrus (worm class). Data from generations I and II (Rd) mice was combined for analysis.

Squares - observed points; vertical bars - 95% confidence limits of the mean counts.

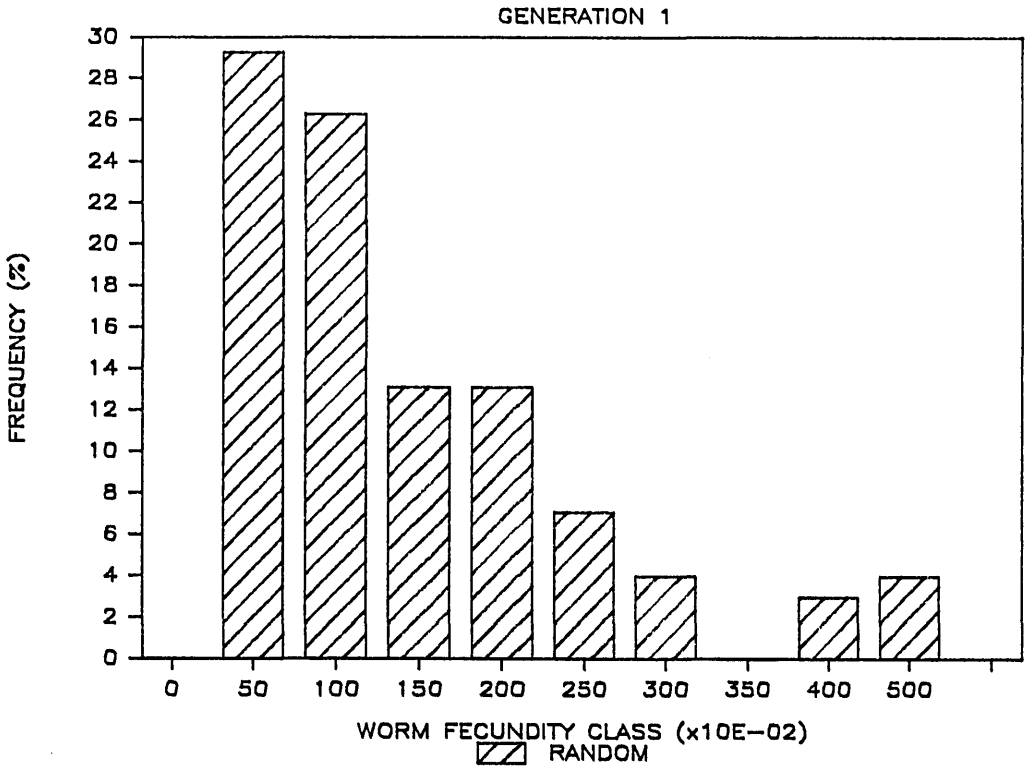
3.4.2 Selection of high and low responder mice

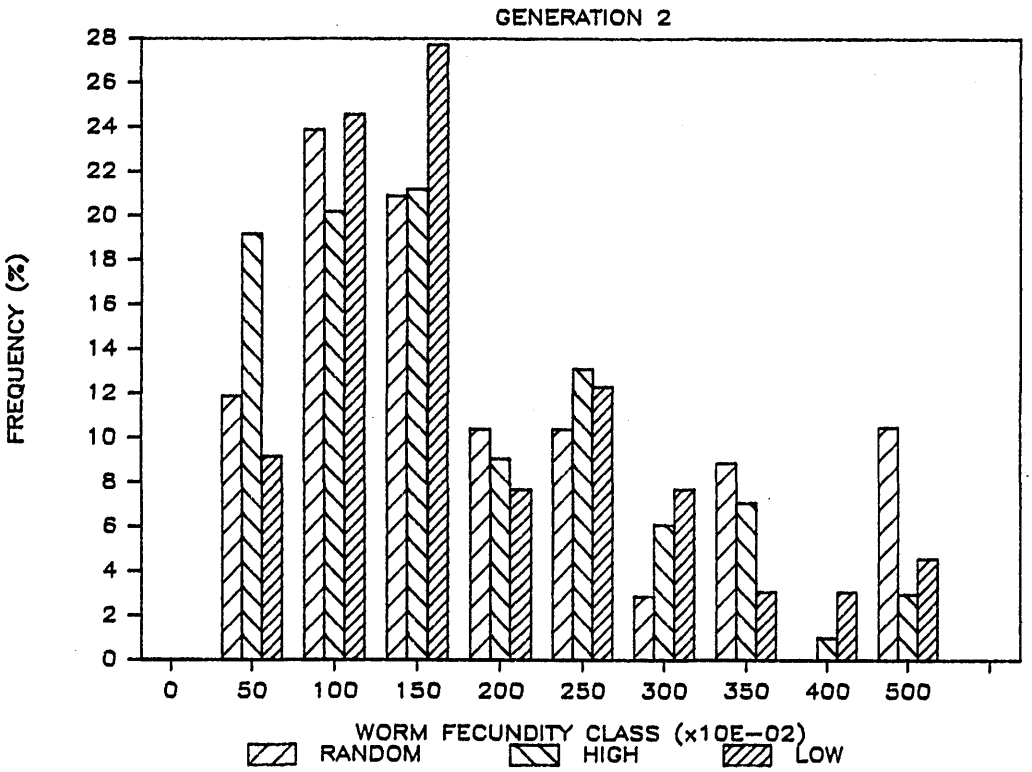
The frequency distribution of parasite eggs/16h in mice of random (Rd), high (H) and low (L) phenotypes from generation I through to generation IV are displayed in Fig. 3.3. These results indicate an asymmetric response of mice to infection with H.polygyrus that became progressively significant as selection progressed (Tables 3.1a and 3.2b). In other words, there was an increasing disparity between high and low responder mice during selection. The variance to mean ratio of the faecal parasite egg counts was increasing among the offspring but stable among selected parents, as selection progressed (Table 3.1b). The control (Rd) population (unselected) maintained a constant proportion of approximately 65% 'high' and 15% 'low' responders (Table 3.2a).

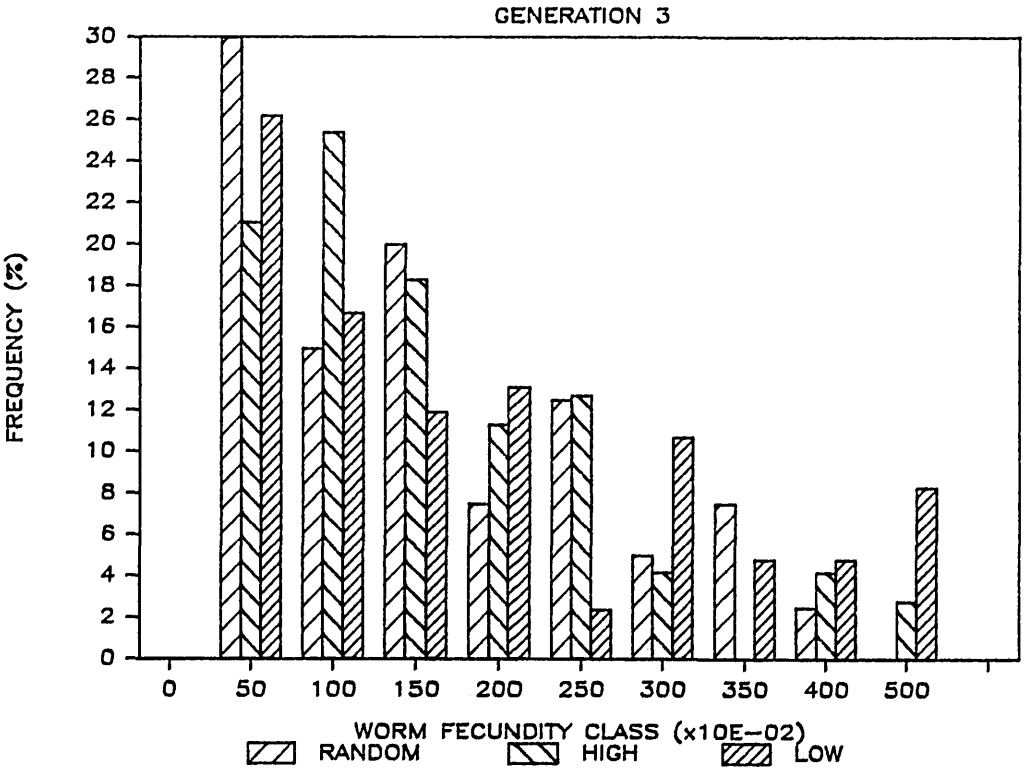
3.4.3 Estimation of the heritability

Theoretical considerations (see 3.2) suggest that it is not desirable to estimate the heritability of a genetic character in relatives subject to selection. Ideally, heritability could be estimated correctly by using a randomly breeding population. Our experimental design, that of keeping the parental Rd mice uninfected, could therefore not permit such an estimate. A crude estimate of the heritability by regression of offspring (II) on parents (I), on the assumption that the gene frequencies would not change substantially was found to be 0.17 ± 0.08 (Table 3.3).

Figure 3.3. The frequency distribution of H.polygyrus eggs per 16h in mice which were selected as high (H) and low (L) responders from a control outbred MF1 random (Rd) population during 4 generations.


GENERATION 1
FREQUENCY (%)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
50
100
150
200
250
300
350
400
500
WORM FECUNDITY CLASS (x10E-02)
RANDOM


GENERATION 2
FREQUENCY (%)
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
50
100
150
200
250
300
350
400
500
WORM FECUNDITY CLASS (x10E-02)
RANDOM
HIGH
LOW


GENERATION 3
FREQUENCY (%)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
50
100
150
200
250
300
350
400
500
WORM FECUNDITY CLASS (x10E-02)
RANDOM
HIGH
LOW

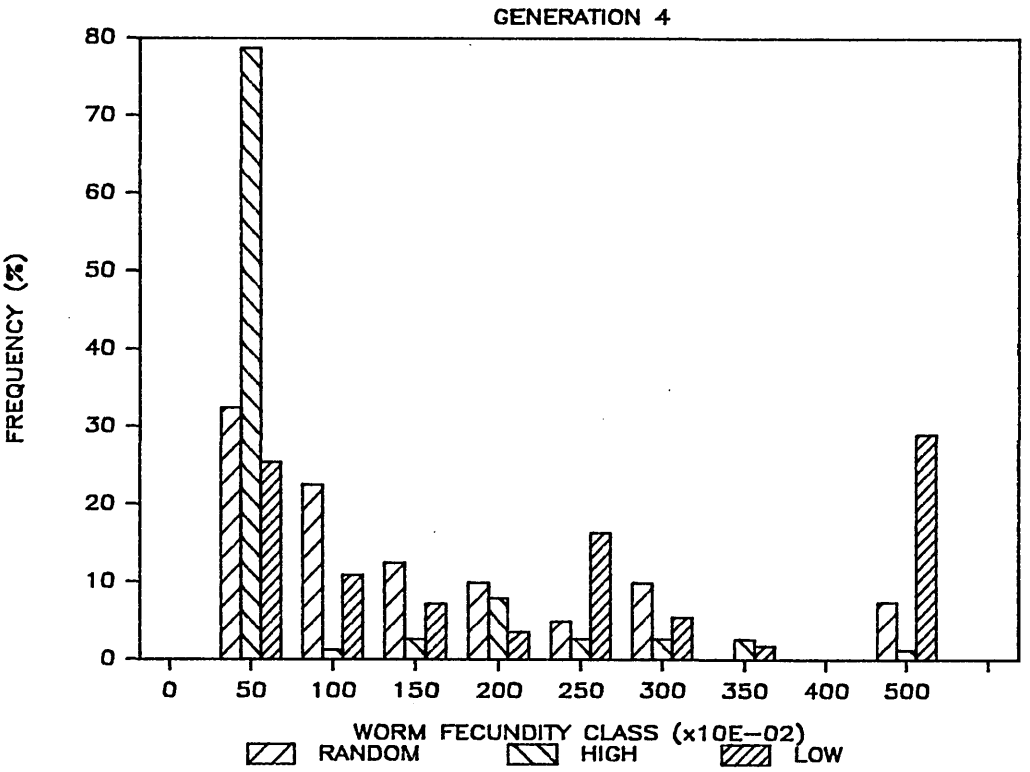

GENERATION 4
FREQUENCY (%)
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
350
400
500
WORM FECUNDITY CLASS (x10E-02)
RANDOM
HIGH
LOW

Table 3.1(a). Summary of the mean parasite eggs/16h/mouse in outbred MF1 (Rd) mice selected for high (H) and low (L) responsiveness to H.polygyrus. (P=number of parents; O = number of offspring-male and female; Gn = generation; n = no.of mice).

Gn		n	(H) Eggs/16h ± s.d.	n	(Rd) Eggs/16h ± s.d.	n	(L) Eggs/16h ± s.d.	
I			-	99	12265 ±11778		-	
II	P	18	1551 ±1645			P 19	31253 ±11876	
	O	99	14979 ±10496	67	17705 ±13674	O 66	16187 ±11840	N.S.
III	P	20	4696			P 20	28548	
	O	71	13399 ±10851	40	12777 ±11142	O 84	17475 ±15918	*
IV	P	20	3771			P 20	38422	
	O	74	4288 ±8434	40	13903 ±14934	O 55	25729 ±24192	***

t-test (H vs L): P>0.05 - Not significant (N.S.)
P<0.05 - * ; P<0.001 *** .

Table 3.1(b). The variance to mean ratio (VMR) of parasite eggs/16h/mouse in Rd, H and L lines during selection of MF1 mice infected with H.polygyrus.

		VMR		
Gn		(H)	(Rd)	(L)
I			11310	
II	P	1745		P 4513
	O	7355	10561	O 8660
III	P	695		P 5434
	O	8788	9716	O 14500
IV	P	1175		P 5692
	O	16589	16041	O 22747

Table 3.2(a). The relative proportion of control MF1 (Rd) mice classified arbitrarily as 'high' ($<15 \times 10^3$ eggs/16h/mouse) and 'low' ($> 25 \times 10^3$ eggs/16h/mouse) responders during selection.

	Proportion (%)	
Generation	High responder	Low responder
I	68.7	11.0
II	56.7	22.3
III	65.0	15.0
IV	67.5	17.5

Table 3.2(b). The relative efficacy of selection for high (H) and low (L) responders from a parental outbred MF1 (Rd) mouse population. (The proportion selected was approximately 20-30%).

	Proportion (%)	
Generation	High responder	Low responder
IV	82.7	52.8

3.4.4 Estimation of the realized heritability

The realized heritability was estimated from the regression of the response to the cumulated selection differential over 4 generations (Table 3.4). The heritability was 0.21 ± 0.29 for the high line; 0.25 ± 0.04 for the low line and 0.26 ± 0.12 for the divergence between the two line. Note the large standard errors of the estimate.

3.4.5 Phenotypic correlation between parasite egg/mouse/16h and mouse litter size

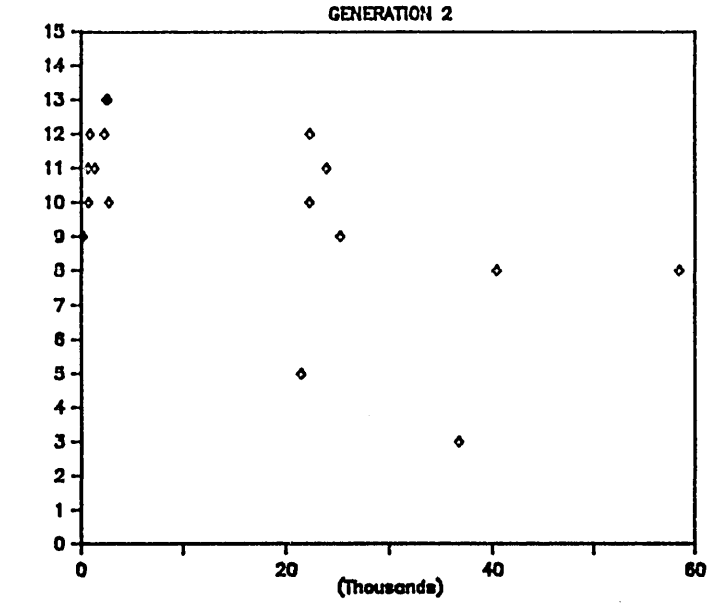
There was a negative correlation between parasite eggs/ mouse/16h and mouse litter size (Fig. 3.4). the Pearson correlation coefficient was only significant in G.II ($P<0.05$) and not significant in G.III and G.IV ($P>0.05$).

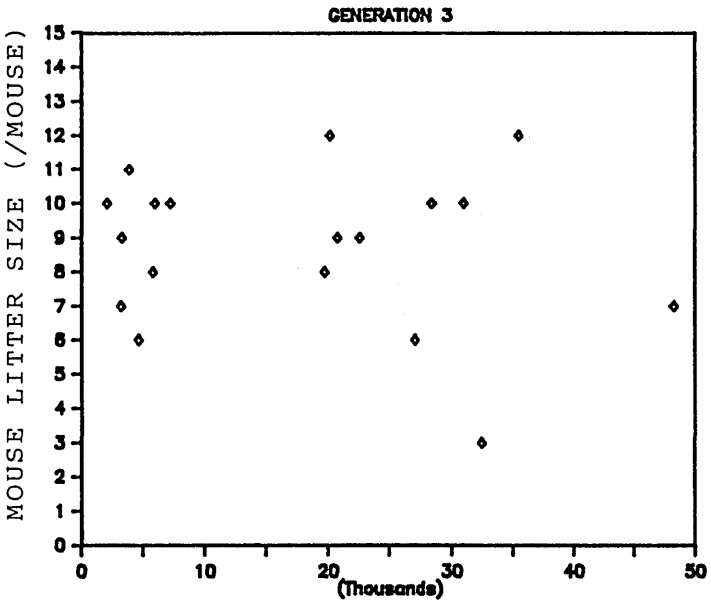
Figure 3.4. The relationship between mouse litter size and the phenotypic character 'faecal parasite eggs/16h/mouse' during selection for high and low responders from outbred MF1 mice.

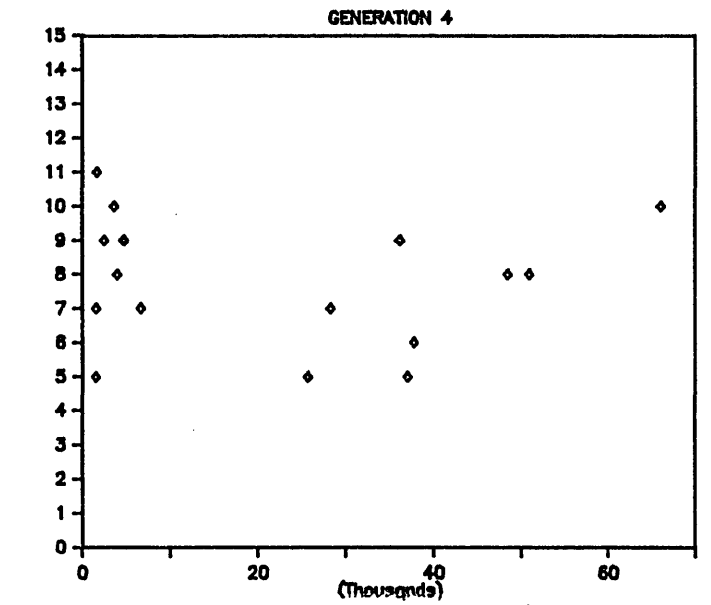
Generation 2: Correlation co-efficient = -0.627; $P<0.05$

Generation 3: Correlation co-efficient = -0.160; $P>0.05$

Generation 4: Correlation co-efficient = -0.082; $P>0.05$.







PARASITE EGGS/16h/MOUSE

Table 3.3. The estimated heritability of the phenotypic character 'faecal parasite eggs/16h/mouse' in outbred MF1 mice. The heritability was estimated from offspring-parent regression (heritability estimates from single parents = x2).

	Offspring - parent regressions ± S.E.		
	Father	Mother	Mid-Parent
Sons	0.151 ± 0.11	0.167 ± 0.12	0.160 ± 0.058
Daughters	0.159 ± 0.15	0.174 ± 0.16	0.167 ± 0.076

Table 3.4. The realized heritability (h^2) estimated from regression of response to selection and cumulative selection differential in outbred MF1 mice infected with 100 L3 H.polygyrus during 4 generations.

Mouse colony	h^2 ± S.E.	r^2	P (α = 0.05)
High	0.206 ± 0.297	0.19	>0.05
Low	0.251 ± 0.043	0.94	<0.05
Divergence (=(H-L))	0.264 ± 0.123	0.69	>0.05

3.5 Discussion

The data presented in Fig. 3.3 and Tables 3.3 and 3.4 suggest that the phenotypic character 'faecal parasite eggs/16h/mouse' is genetically acquired between parental mice and their offspring. This data can be interpreted as a demonstration of the genetic control of parasitic nematode, H.polygyrus, by an outbred laboratory mouse strain.

Selection of mice from a base population of outbred MF1 mice revealed an asymmetrical response which separated, as selection progressed, mice excreting many parasite eggs/16h from those excreting fewer parasite eggs/16h. Based on the positive correlation between faecal parasite eggs/16h and the number of female worms recovered (Fig. 3.1), it must be concluded, therefore, that mice belonging to the first category i.e. excreting many parasite eggs are low responders or innately more susceptible than the high responders (those excreting fewer parasite eggs per 16h). A relatively high realized heritability of 25% confirms this observation. This value differs from the heritability estimates of 45-55% reported by Brindley & Dobson (1981) and Sitepu & Dobson (1982), but is similar to the estimate (by sib-analysis) reported by Brindley & Dobson (1983). The standard errors associated with these estimates are too high to show any differences between the separate estimates (Hill,1971).

Genetic control of H.polygyrus in F1(Quackenbush x wild) mice is believed to be under the control of 2 or 3 genes (Brindley & Dobson,1981). The results reported here confirm Brindley & Dobson's (1981) data based on eggs per gram of faeces. Data based on eggs/gram faeces can be unreliable in the assessment of the intensity of intestinal parasitic infections due to the fluctuations in host faecal output and rhythmic parasite egg deposition (see Lewis & Shava,1977; Kerboeuf & Jacob,1983; Anderson & Schad,1985; Keymer & Hiorns,1986). To increase accuracy, it is desirable to collect faeces from mice over at least 12h (Kerboeuf & Jacob,1983).

The limited data shown here suggests that an outbred laboratory mouse (MF1) population is genetically heterogeneous, consistent with theoretical expectation (Rice & O'Brien,1980). However, the use of small numbers of mice may lead to differing population dynamic patterns since the proportion of high and low responders may vary between different experiments (see Vaupel & Yashin,1985).

The genetic control of parasitism has far-reaching implications both for the evolutionary relationships between host and parasite (Price,1980; Holmes,1982; Wakelin,1985a), animal productivity (Dargie,1982) and for the control of parasitic infections (Anderson & May, 1982; 1985a). The interaction between host fecundity and host responsiveness reflect a balance between the gain

associated with high host fecundity and the cost of being hyper-responsive (Cohen,Altmann & Friedman,1985). Moreover, transmission of resistant or susceptible genes in natural mouse populations may be affected by the relationship between behavioural dominance and territoriality (Wilson,1975). Parasites may alter the dominance-subordinate relationships in mice (Freeland,1981; Rau,1983a,b; 1984). Thus, there exists a possibility that resistant and therefore, dominant mice, may be at a reproductive advantage over the heavily infected subordinate mice (DeFries & McClearn,1970). Random drift may be important because of the small size of effective breeding mouse colonies (Reimer & Petrus,1967).

Chapter 4

Single and challenge infections

4.1 Introduction

Many experimental models of human hookworm infection, including self infections, have been suggested. The outbred mouse-*H.polygyrus* system has been widely used in the screening of anthelmintics (Jenkins,1982; Ibarra & Jenkins,1984), in nutritional (Bawden,1969; Slater & Keymer,1986), physiological (Croll *et al*,1981), immunological (Panter,1969; Cypess *et al*,1976; Bartlett & Ball, 1974; Day *et al*,1980; Pritchard, Williams & Behnke,1983; Ali & Behnke,1985), genetic (Behnke & Wakelin,1977; Prowse *et al*,1979; Brindley & Dobson,1982; Behnke & Robinson,1985) and other experimental studies described in chapter 1. *Nippostrongylus brasiliensis* in rats has also been employed as a model of hookworm infection (Kassai,1981) but has the disadvantage that, unlike human hookworm, the infection is not chronic.

Other laboratory models include the dog-*Ancylostoma caninum* system which has been used, *inter alia*, in the development of a vaccine for dog hookworm (Miller,1971;1978) and to investigate the biochemical properties of the parasite's proteolytic enzymes (Hotez *et al*,1985). *Necator americanus* in neonatal hamsters, has also been used (Sen & Seth,1967; Behnke, Paul & Rajasekariah,1986; Carr & Pritchard,1986). Recently, the human strain of *A.ceylanicum* in dogs has provided a useful model of human hookworm infection and is thought to be likely to improve the prospects for the development of a human hookworm vaccine (Carroll & Grove,1984;1985). However, financial

costs, difficulty in handling and ethical issues prohibit the widespread use of dogs as experimental models.

Mouse-H.polygyrus models are particularly amenable to laboratory investigation because of the relatively short generation time of the parasite, the ease of maintenance, availability and genetic characterization of mice (Festing,1982; Wakelin,1982). Thus, if the purpose of an experiment is to mimic the transmission of hookworm in natural populations, outbred mice may be used with the assumption that such mice are genetically heterogeneous (Rice & O'Brien,1980). Theoretically, one would expect a low degree of genetic heterogeneity in inbred mice and therefore, similar responses between individuals (Festing, 1982; Green,1981), but, their homogeneity has been questioned recently (Bailey,1982).

Despite the wealth of information available at present, quantitative estimates of the parameters determining the transmission of H.polygyrus in mice remains limited. Knowledge of such parameter estimates is essential to the interpretation and analysis of parasite population growth and transmission. The main aim of this chapter is, therefore, to describe simple, single infection experiments in order to obtain initial parameter estimates. These estimates will be used in the analysis of more complex repeated infection experiments. A diagrammatic flow chart of the population processes involved in the transmission of H.polygyrus is shown in Fig. 4.1 and the relevant rate-

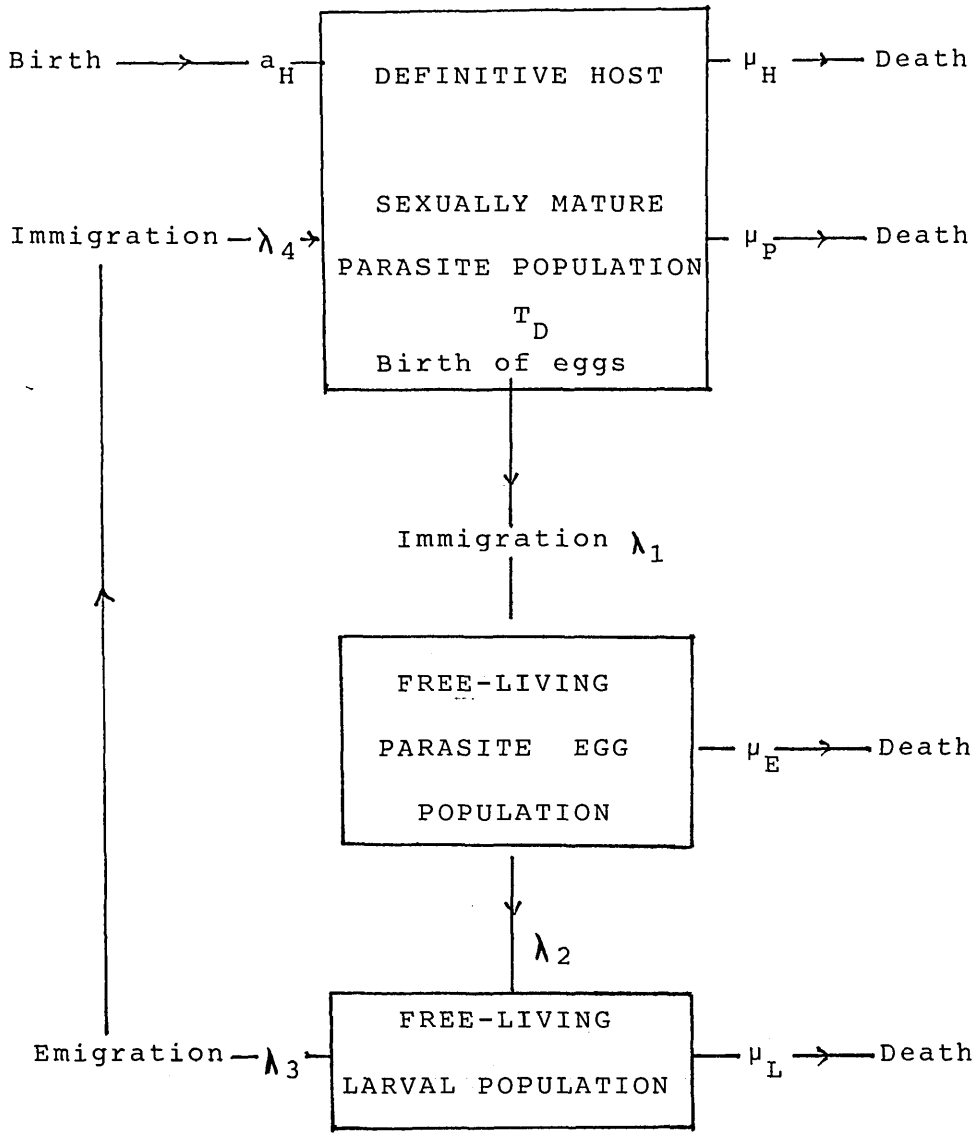


Figure 4.1. Diagrammatic representation of the direct life-cycle trichostrongyloid nematode parasite, Heligmosomoides polygyrus showing the various rate-determining population parameters controlling the transmission of the parasite (After Anderson,1976).

determining parameters are displayed in Table 4.1.

The importance of biological processes in the regulation of host and parasite populations (e.g. the age- and density-dependent regulation of parasite growth, fecundity and survivorship) has already been emphasized (Anderson & May,1978; May & Anderson,1978; Anderson,1978). Anderson (1976) has suggested that an ecological appraisal of host-parasite populations and a better understanding of their dynamical properties may help to improve approaches to community-based control of helminth infections (Anderson & May,1982).

This chapter describes single infection (=primary) experiments and the variability inherent in the larval estimation technique is examined (4.2.1). Also, the relationship between the dose of infective larvae and the establishment of adult worms (4.2.2); the survivorship characteristics of these established worms (4.2.3.1); their reproductive rate over time and their dependence on age and density of the parasite population (4.2.3.2), are investigated. In addition, the significance of the level of primary infection on the subsequent acquisition of graded challenge infections, following chemotherapeutic clearance of existing primary infections, is assessed(4.3). The results are discussed (4.4) and their relevance to the overall dynamics of host-parasite relationships is briefly considered.

Table 4.1. Estimates of the various population rate-determining parameters in the transmission of H. polygyrus (/parasite or host/ unit of time).

Parameter	Symbol	Value	References
Instantaneous birth rate of host	a_H		
Instantaneous death rate of host	b_H		
Instantaneous parasite-induced host mortality rate	μ_H	10^{-3} - 4×10^{-4}	Forrester(1971); Keymer & Hiorns(1986); this study.
Instantaneous birth rate of parasite eggs per day	λ_1	500 - 1200	this study.
Instantaneous birth rate of larvae	λ_2	-	-
Rate of parasite establishment in host	λ_4	0.45 - 0.66	this study
Time to maturity in host	T_D	8-10 d	Bryant(1973) Bartlett & Ball(1974).
Instantaneous rate of parasite mortality	μ_P	0.03 - 0.10	this study; Keymer & Hiorns(1986).
Average life-span of the parasite	$1/\mu_P$	9-35 wks	this study Keymer & Hiorns(1986).
Maximum life-span of parasite	T_P	32 wks	Ehrenford(1954)
Instantaneous rate of mortality of the free-living parasite egg population	μ_E	-	-
Instantaneous rate of mortality of free-living larval population	μ_L	-	-
Duration of acquired resistance	$1/\sigma$	>50 wks	Van Zandt et al(1973).
Negative binomial	k	-	Lewis(1968a)($s^2>x$)
Sex ratio		approx.1:1	this study
Mating habits		polygamous?	

4.2 Results from single infection experiments

4.2.1 Estimation of the number of infective larvae

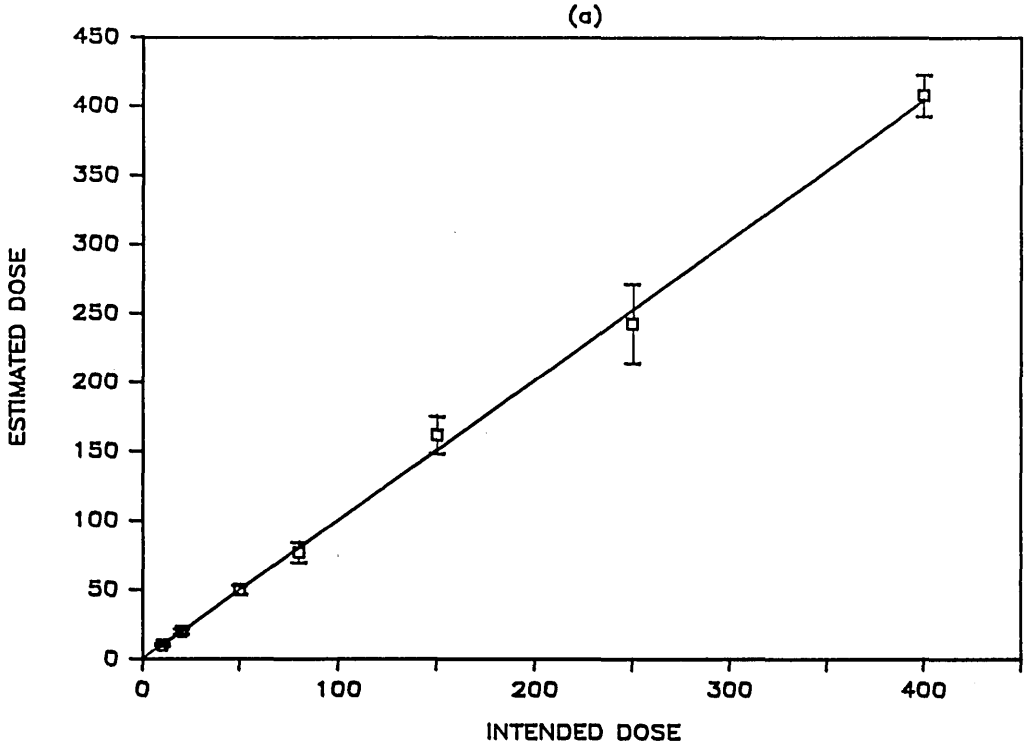
The accuracy of the estimated infective larval dose was assessed by counting the number of larvae per 0.1 ml from a stock suspension of a known volume, in 10 replicates, under a dissecting microscope. The suspension was then diluted to give the required number of larvae/0.1ml (intended dose). Sample 0.1ml volumes (n=10) were then dispensed and the total number of larvae counted to obtain an 'estimated dose'. The results in Fig. 4.2(a) show that the accuracy of the intended dose was very high (r^2=0.998). The variance of the mean counts increased with increasing counts (b=1.93)(Fig. 4.2b).

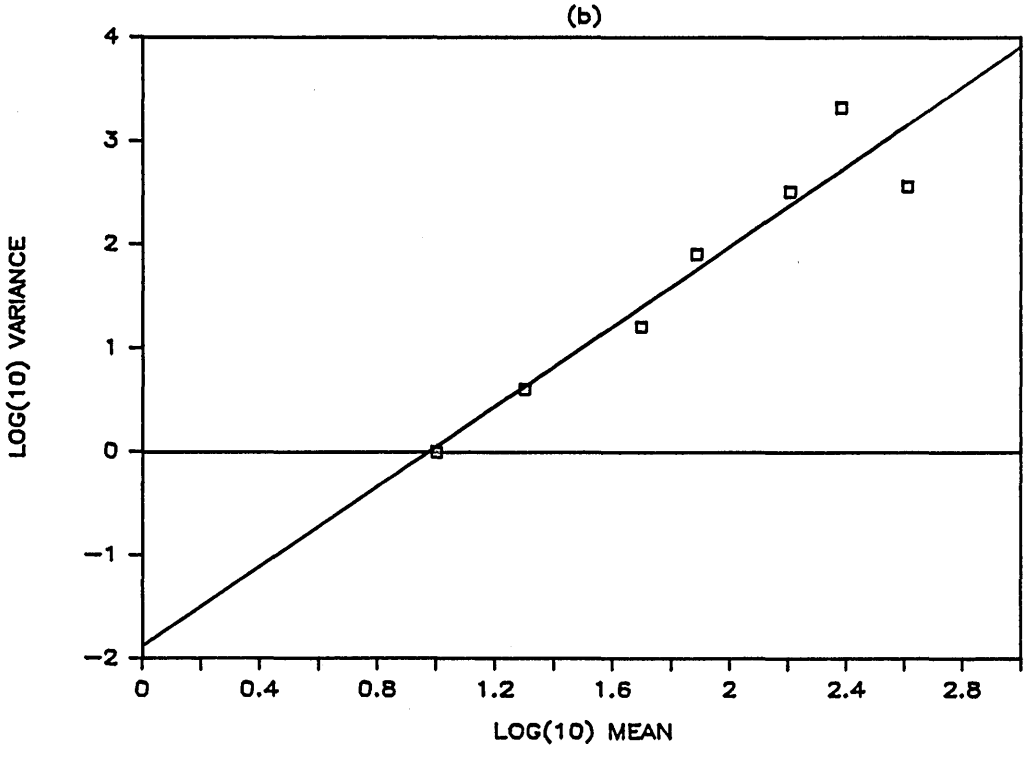
There are several methods for measuring the variability in a measurement (Elliott,1977; Southwood,1978; Taylor,1961). In general, the degree of overdispersion or aggregation can be estimated from the departure of the variance to mean ratio from unity. The distribution will be aggregated if $s^2/\bar{x} > 1$; random (Poisson) if $s^2/\bar{x} = 1$ and uniform (underdispersed), if $s^2/\bar{x} < 1$ (Elliott,1977). Also, the parameter, k, of the negative binomial probability distribution provides an inverse measure of overdispersion (= aggregation, heterogeneity or contagion)(Bliss & Fisher,1953). An empirical relationship between the variance (s^2) and the mean ($\bar{x}$) for

Figure 4.2. The relationship between the 'intended' and 'estimated' dose of infective H.polygyrus larvae.

(a) Squares - observed mean number of larvae/0.1 ml; vertical lines - 95% confidence limits (n=10); solid line - best fit linear model constrained through the origin (y=bx). Parameter values: b = 1.0118; r^2 = 0.998.

(b) Squares - the log(10) variance plotted against the log(10) mean; solid line - best fit linear model of the form y = a + bx where a = -1.8808; b = 1.9306; r^2 = 0.904.


(a)
ESTIMATED DOSE
INTENDED DOSE
0
50
100
150
200
250
300
350
400
450
0
100
200
300
400


(b)
LOG(10) VARIANCE
LOG(10) MEAN
4
3
2
1
0
−1
−2
0
0.4
0.8
1.2
1.6
2
2.4
2.8

ecological data is given by Taylor (1961), where $s^2 = a \bar{x}^b$. A plot of log(10) variance against log(10) mean gives a slope, b, and the y-intercept is equal to log(10) a. For b=1, the distribution is Poisson (random) and if b>1 , the pattern is aggregated.

4.2.2 Establishment of adult worms

The rate of establishment of adult worms in hosts depends on several factors such as the strain, sex and age of host, the number of infective stages and the infection technique. The rate of establishment of adult H.polygyrus in outbred and inbred mice was assessed by post-mortem dissection of mice 14 days post-infection with larvae.

Seventy outbred female MF1 mice were infected, in groups of 8-10 mice, at 6-8 weeks of age with 20, 40, 100, 160, 300, 500 and 800 infective H.polygyrus larvae per mouse. Fourteen days after infection, the number of worms in these mice were counted following a 16h faecal egg collection (see 4.2.3). Similarly, 55 female, 6-8 week old, inbred CBA/Ca mice were divided into groups of 6-10 mice and infected with 50, 100, 200, 300, 400 and 500 H.polygyrus larvae per mouse; 41 female, 6-8 week old, inbred NIH mice were divided into groups of 5-9 mice and infected with 10, 25, 50, 100, 230 and 450 H.polygyrus larvae per mouse and their worm burdens determined 14 days after infection

The results in Fig. 4.3 (a,b,c) show the rate of establishment of H.polygyrus in the three strains of mice. On average, 44% of the worms established in outbred female MF1; 66% established in inbred female CBA/Ca and 57% established in inbred female NIH mice. Within the experimental ranges employed in these studies, the establishment of H.polygyrus was related directly to the density of infective larvae. However, a high degree of variability with increasing doses of infection was observed in the rate of establishment of H.polygyrus in all strains of mice (Fig. 4.4 a,b,c).

On average, the adult worm sex-ratio (female/total) was biased towards a preponderance of female parasites in all three strains of mice (Table 4.2). Sex ratio analyses and their significance levels are presented in Table 4.2 (a). A depature from the 1:1 sex ratio was observed at the high doses of infection. Although the rate of parasite establishment was found to be dose-independent in this study (Fig. 4.3), a heterogeneity chi-square analysis (Zar,1984) indicated that it would not be justifiable to pool all the data (over all doses of infection)(heterogeneity in variance) for CBA, but not for NIH and MF1 mouse strains (Table 4.2b).

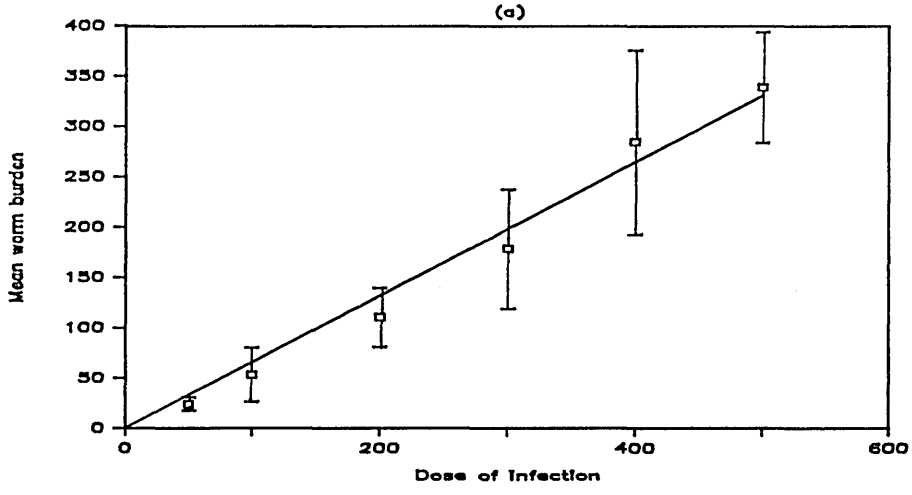
Figure 4.3. The observed mean worm burdens in inbred CBA/Ca and NIH and outbred MF1 mice, 14 days after infection with varying doses of H.polygyrus larvae.

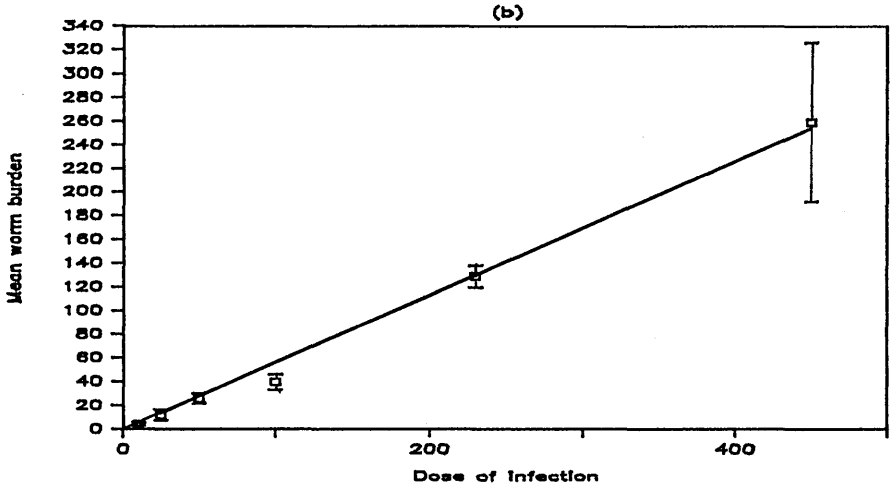
Squares - observed worm burdens in (a) CBA/Ca, (b) NIH and (c) MF1 mice; vertical bars - 95% confidence limits; solid lines - best fit linear models of the form $y = bx$ (constrained through the origin). Parameter value:

(a) $b = 0.66$; $r^2 = 0.9897$;

(b) $b = 0.57$; $r^2 = 0.9958$;

(c) $b = 0.44$; $r^2 = 0.9166$.


(a)
Mean worm burden
400
350
300
250
200
150
100
50
0
0
200
400
600
Dose of Infection


(b)
Mean worm burden
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
0
200
400
Dose of Infection

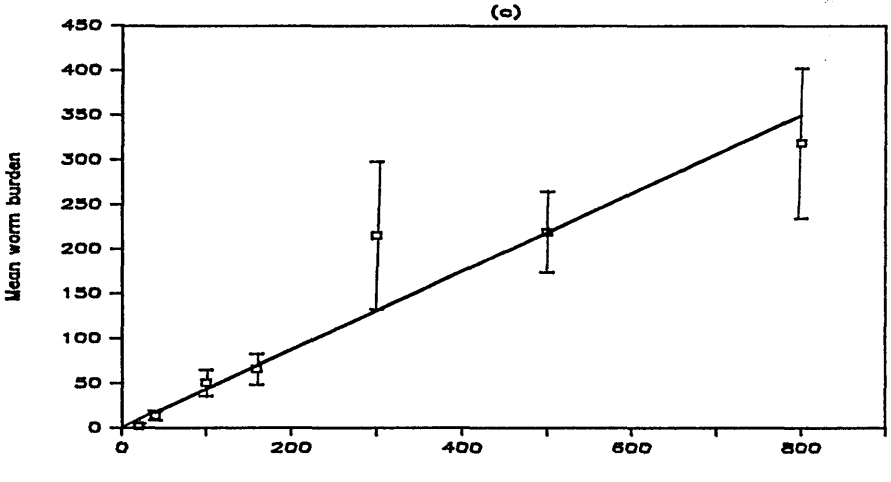

(c)
Mean worm burden
450
400
350
300
250
200
150
100
50
0
0
200
400
600
800

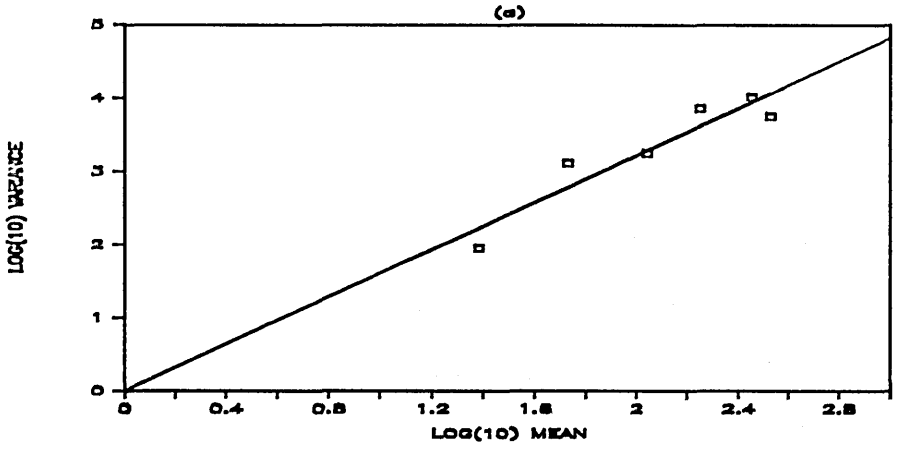
Figure 4.4. A plot of the log(10) variance against the log(10) mean worm burden in inbred CBA/Ca, NIH and outbred MF1 mice, 14 days after infection with H.polygyrus.

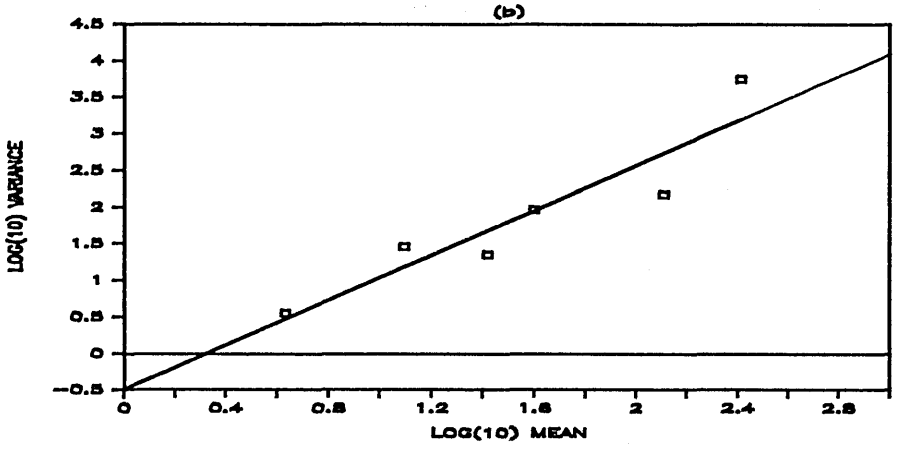
Solid lines - best fit linear models of the form $y = a + bx$, with parameter value:

(a) CBA/Ca: $a = 0.0029$; $b = 1.6082$; $r^2 = 0.8799$;

(b) NIH : $a = -0.4935$; $b = 1.5314$; $r^2 = 0.8606$;

(c) MF1 : $a = 0.1759$; $b = 1.5641$; $r^2 = 0.9601$.


(a)
LOG(10) VARIANCE
0
1
2
3
4
5
0
0.4
0.8
1.2
1.6
2
2.4
2.8
LOG(10) MEAN


(b)
LOG(10) VARIANCE
-0.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0
0.4
0.8
1.2
1.6
2
2.4
2.8
LOG(10) MEAN

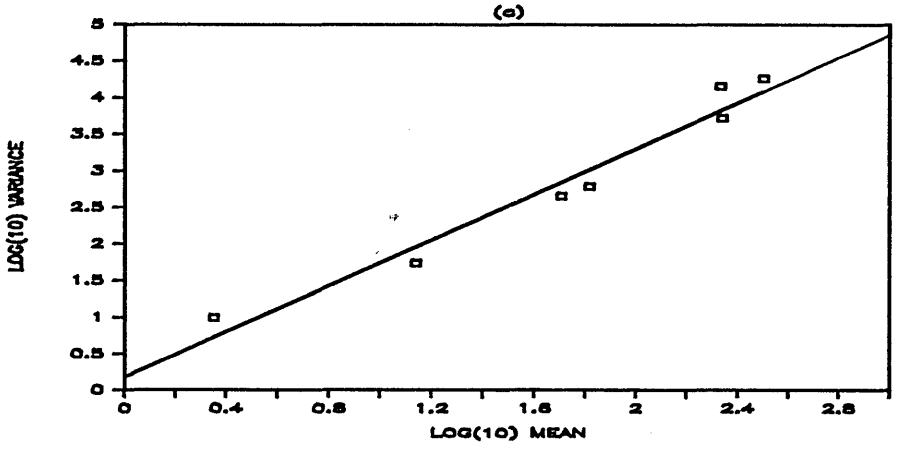

(c)
LOG(10) VARIANCE
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
0
0.4
0.8
1.2
1.6
2
2.4
2.8
LOG(10) MEAN

Table 4.2(a). Analysis of worm sex ratios in inbred CBA/Ca, NIH and outbred MF1 mice infected with varying single doses of H.polygyrus. The establishment of worms was assessed 14 days after infection.

NIH				CBA/Ca				MF1			
dose	F/T	X^2*	P	dose	F/T	X^2	P	dose	F/T	X^2	P
10	0.57	0.30	>.50	50	0.53	0.59	>.25	20	0.50	0.06	>.75
25	0.59	1.92	>.10	100	0.50	0.002	>.95	40	0.54	0.58	>.25
50	0.50	0.008	>.90	200	0.52	1.93	>.10	100	0.57	7.71F	<.01
100	0.57	6.67F	<.01	300	0.54	10.7F	<.01	160	0.57	10.4F	<.005
230	0.57	16.5F	<.001	400	0.46	8.44M	<.01	300	0.56	26.3F	<.001
450	0.52	2.87F	>.05	500	0.52	4.56F	<.05	500	0.55	19.6F	<.001
								800	0.56	38.6F	<.001

* bias in sex-ratio (F= female; M= male; T= total worms)

H_o: sex ratio (F:M) = 1:1

H_A: sex ratio = 1:1

P = probability (α = 0.05)

Table 4.2(b). Heterogeneity chi-square analysis of the data in Table 4.2(a) from inbred CBA/Ca, NIH and outbred MF1 mice.

NIH		D.F.	CBA/Ca	D.F.	MF1	D.F.
Total	X^2=28.217	6	26.199	6	103.164	6
X^2_{total}	=22.696	1	4.733	1	102.438	1
X^2 (hetero-geneity)	= 5.521	5	21.466	5	0.726	5
	0.25<P<0.50		P<0.001		0.975<P<0.99	

4.2.3 Age- and density-dependent worm survivorship and fecundity

4.2.3.1 Estimation of parasite mortality

Single infection experiments in which hosts are exposed once to infective parasitic stages are helpful in the assessment of parasite surviviorship over time. Survivorship can be determined by destructive sampling at intervals from an initial cohort of hosts all infected at the same point in time. The assumptions being that such parasites do not replicate within the host; there is no immigration or recruitment of the parasite and provided they are chosen at random, the samples will be representative of the orginal population.

Three experiments, using three different mouse strains, were conducted. One hundred and fifty inbred, female 6-8 week old CBA/Ca mice were divided into 4 groups of 36 mice each. Each group of mice was infected with 50, 100, 200 and 400 H.polygyrus larvae per mouse on day 0. At about 2-weekly intervals, 6 mice from each group were selected at random and killed to determine their worm burdens following faecal egg collection. Similarly, 40 inbred, female 6-8 week old NIH mice, in two groups of 20 mice each, were infected with 50 and 100 H.polygyrus larvae per mouse. This was repeated with 105 outbred, female 6-8 week old MF1 mice in three groups each infected with 100, 200 and 400 H.polygyrus larvae per mouse.

The results are shown in Fig. 4.5 and Table 4.3. The mean worm burdens decreased slowly suggesting that within the experimental period employed in this study, the rate of parasite mortality was low. The variance to mean ratios (VMR) indicated that the parasite's distribution was overdispersed, especially in outbred MF1 mice (Table 4.3). The proportion of female worms was always high in inbred mice throughout the experiment, whereas more male worms were recovered in outbred mice during the latter part of the infection (Table 4.3).

Direct measurement of the rate of parasite mortality is made difficult by the high degree of variability between mice recorded in the experiments. Our estimates of the death rate (μ) rely on worm survivorship data as has been done for various organisms (Anderson & Whitfield,1975; Asch & Drane,1972; Garg, Rao & Redmond,1970; Smith & Grenfell,1984). Assuming that the number of worms surviving at different times during the course of an infection can be described by a pure death process (there is no immigration of infective H.polygyrus larvae other than at the beginning of the experiment), a deterministic model (Anderson & Whitfield,1975) can be used to describe the change in N_t, the mean number of parasites surviving at time, t:

$$\frac{dN_t}{dt} = -\mu N_t \qquad [4.1]$$

where μ = the instantaneous death rate per parasite per unit of time (assumed to be constant throughout the parasites's life-span).

Figure 4.5. The mean number of adult H.polygyrus surviving over a period of up to 24 weeks following a primary infection in inbred CBA/Ca mice.

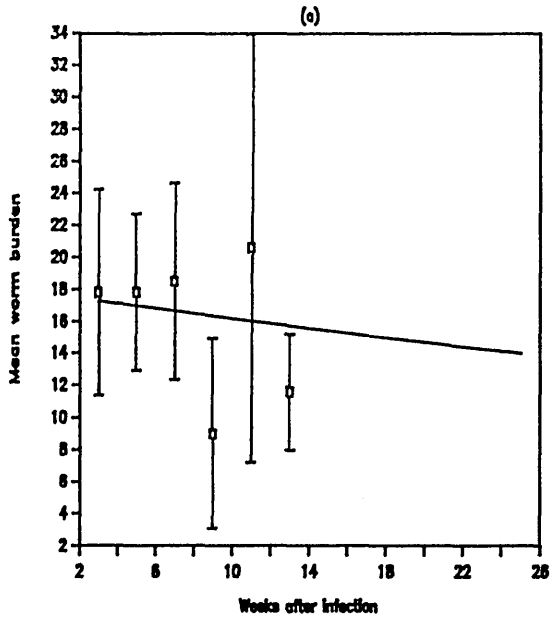
Squares - observed points (n= 6-10); solid lines- best fit exponential models of the form $N(t) = N(0) \exp(-\mu t)$; vertical bars - 95% confidence limits. Parameter values:

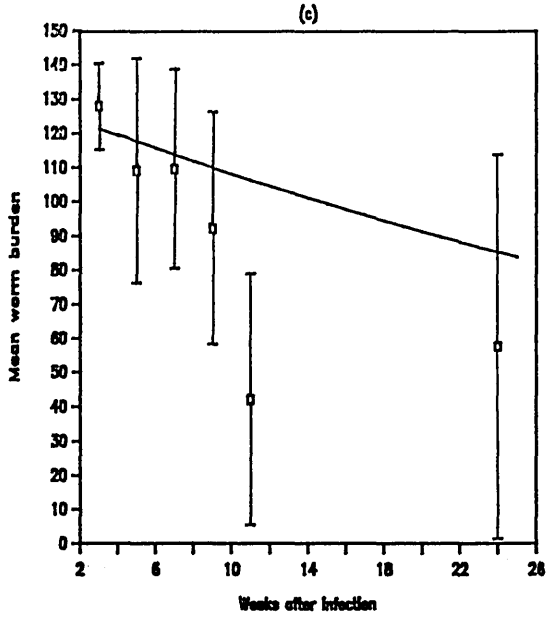
(a) Dose=50L3; ln N(0)=2.881; μ(±s.e.)=0.0098 ± 0.029;

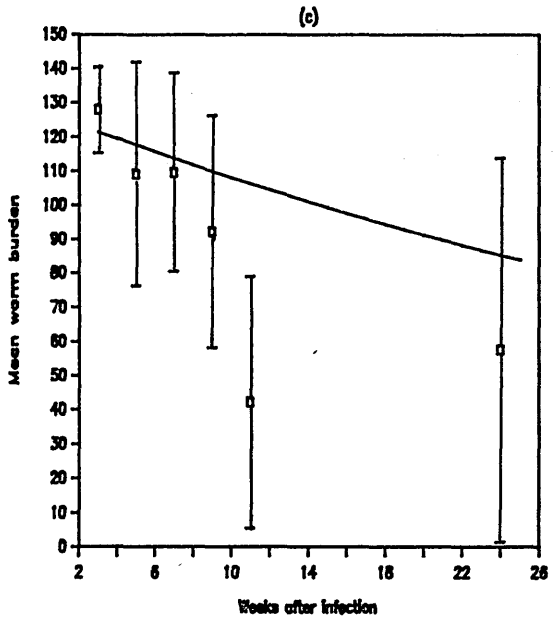
(b) Dose=100L3;ln N(0)=4.290; μ(±s.e.)=0.024 ± 0.024;

(c) Dose=200L3;ln N(0)=4.852; μ(±s.e.)=0.024 ± 0.017;

(d) Dose=400L3;ln N(0)=5.651; μ(±s.e.)=0.054 ± 0.071.


(a)
Mean worm burden
Weeks after infection


(c)
Mean worm burden
Weeks after infection


(c)
Mean worm burden
Weeks after infection

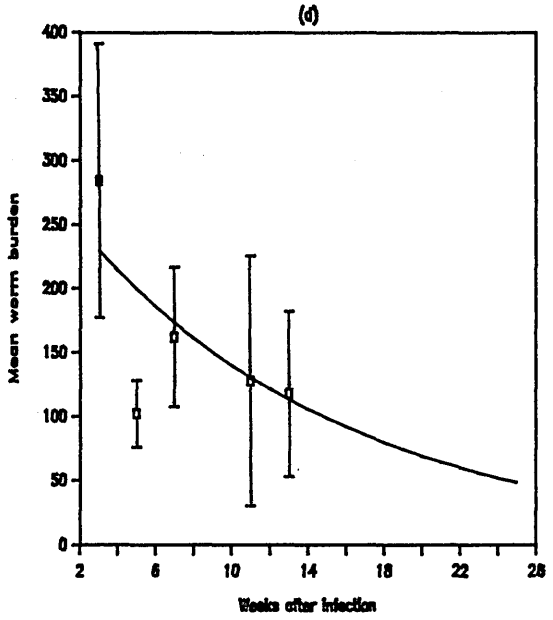

(d)
Mean worm burden
Weeks after infection

Table 4.3. The mean number of surviving adult H.polygyrus following single infection of outbred MF1 and inbred CBA/Ca and NIH mice with varying initial doses of infection.

MF1

	dose = 100 L3				dose = 200 L3				dose = 400 L3			
week	n	MWB ±se	V/M	F/T	n	MWB ±se	V/M	F/T	n	MWB ±se	V/M	F/T
4	6	77.83 ±17.0	22.3	0.57	6	136.33 ±24.9	27.2	0.52	6	214.0 ±45.8	58.8	0.56
8	6	34.33 ±6.45	7.3	0.67	6	83.50 ±24.4	46.7	0.58	6	91.8 ±17.3	19.7	0.55
12	6	10.17 ±9.8	56.3	0.29	6	23.00 ±22.2	128.7	0.39	6	43.2 ±27.6	105	0.43
16	8	0.87 ±0.7	5.0	0	8	8.75 ±6.6	40.4	0.38	6	9.5 ±9.5	57.1	0.19
20	6	0	-	-	6	0	-	-	6	0	-	-
24	10	0	-	-	9	1.44 ±1.4	13.0	0	10	9.2 ±5.2	29.4	0.12
28	10	0	-	-	10	0	-	-	8	9.4 ±9.4	74.9	0.16

NIH

	dose = 50 L3				dose = 100 L3			
week	n	MWB(±s.e.)	V/M	F/T	n	MWB(±s.e.)	V/M	F/T
2	5	22.4±11.4	28.8	0.50	5	33.8±6.9	7.1	0.56
6	5	6.0±1.8	2.7	0.97	5	34.6±7.1	7.3	0.63
9	5	9.2±0.7	0.2	0.74	5	4.8±1.2	1.4	0.75
12	5	5.0±2.6	6.5	0.84	5	2.0±0.8	1.7	1.00

Table 4.3 (continued)

CBA/Ca

dose = 50 L3					dose = 100 L3			
week	n	MWB±(s.e.)	V/M	F/T	n	MWB(±s.e.)	V/M	F/T
3	6	17.83±2.5	2.1	0.54	6	73.00±9.2	6.9	0.48
5	6	17.83±1.9	1.3	0.53	6	53.33±11.3	14.5	0.49
7	6	18.50±2.4	1.9	0.59	6	35.50±7.6	9.7	0.56
9	6	9.00±2.3	3.6	0.54	6	47.83±12.5	19.6	0.56
11	6	20.60±5.2	6.6	0.52	6	63.00±19.2	29.3	0.55
13	6	11.60±1.4	0.9	0.64	6	41.00±8.7	7.4	0.59

dose = 200 L3					dose = 400L3			
week	n	MWB(±s.e.)	V/M	F/T	n	MWB(±s.e.)	V/M	F/T
3	6	128.00±4.9	1.2	0.54	6	284.50±41.6	36.5	0.46
5	6	109.17±12.8	8.9	0.57	6	102.33±10.0	5.9	0.51
7	6	109.67±11.3	6.9	0.54	6	162.33±21.2	16.6	0.42
9	6	92.17±13.2	11.3	0.55		-	-	-
11	6	42.20±14.3	29.0	0.53	6	128.20±37.9	56.3	0.49
13		-	-	-	6	117.80±25.9	28.7	0.48
24	6	57.67±21.9	49.6	0.37		-	-	-

MWB = mean worm burden
V/M = variance to mean ratio
F/T = ratio of female worms to total worms
n = number of mice used
s.e.= standard error of the mean

Given that there are N_o worms at the start of the infection (t=0), equation [4.1] has the following solution:

$$N_t = N_o \exp(-\mu t) \qquad [4.2]$$

This equation [4.2], describes simple exponential decline in population size. Based on the assumption that 'μ' is approximatedly constant during one small time interval, it is possible to estimate $\mu(N,t)$, the age- and density-dependent instantaneous parasite mortality rate (see Anderson & Whitfield,1975; Anderson,1976; Mills, Anderson & Whitfield,1979). Parasite death rate over a defined unit of time, μ_t, can be estimated from the equation,

$$\mu_t = \frac{\ln N_t - \ln N_{t+1}}{t_{0.5}} \qquad [4.3]$$

The relationship between μ and time (weeks) is shown in Fig. 4.6. It is clear that at all initial parasite densities tested in this experiment, the slopes (b) of the best-fit linear model ($\mu=\mu_o+bt$, where b and μ_o are constants) are not significantly different from zero ($F(a)=0.658, P>0.25$; $F(b)=1.118, P>0.25$; $F(c)=0.541, P>0.25$; $F(d)=0.808, P>0.25$) and therefore the death rate of the parasite can be assumed to be constant over a period of up to 24 weeks. The estimated life-span of *H.polygyrus* ($1/\bar{\mu}$) was 9.9 weeks, 8.9 weeks and 35.8 weeks in MF1, NIH and CBA/Ca mouse strains, respectively (Table 4.4).

Figure 4.6. The instantaneous parasite death rates (per parasite per week) in inbred CBA/Ca mice infected with varying initial densities of infective H.polygyrus larvae.

Squares - observed points; solid lines - best fit linear models of the form $\mu = a + bt$.

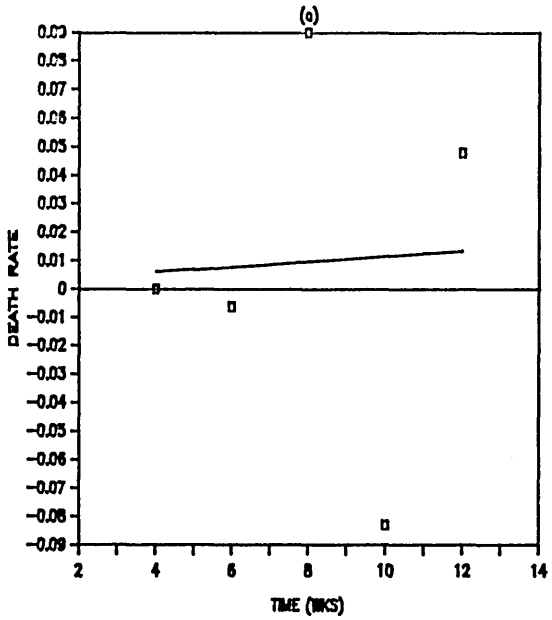
Parameter values:

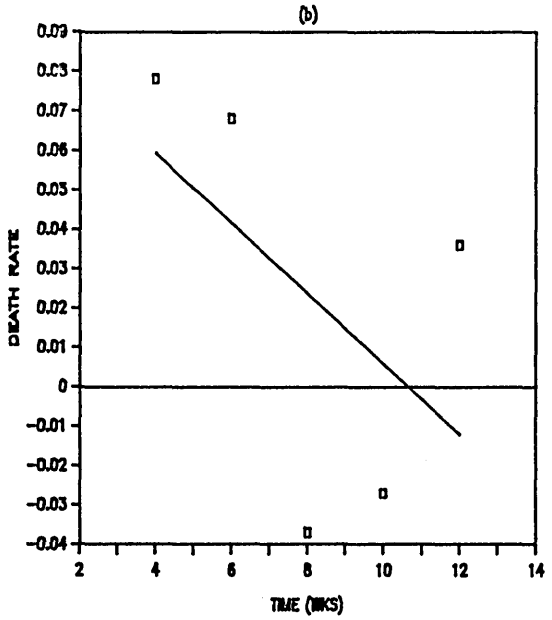
(a) N(0)= 17.83; a = 0.0021; b = 0.00096;

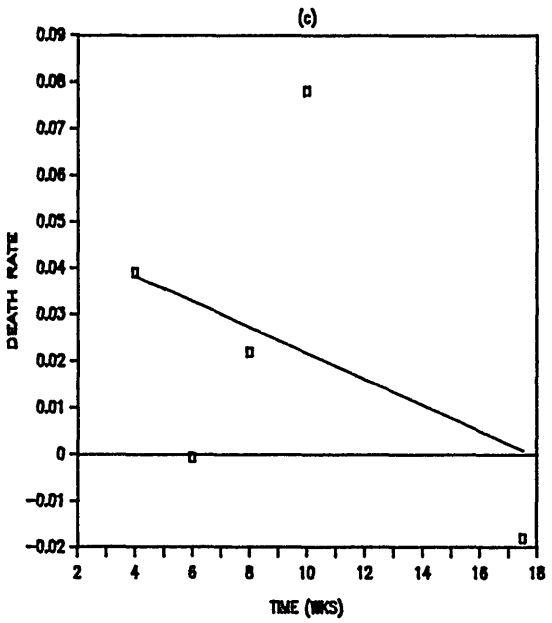
(b) N(0)= 73.00; a = 0.0952; b =-0.00895;

(c) N(0)= 128.00; a = 0.0495; b =-0.00279;

(d) N(0)= 284.50; a = 0.2393; b =-0.0255.


(a)
DEATH RATE
TIME (WKS)


(b)
DEATH RATE
TIME (WKS)


(c)
DEATH RATE
TIME (WKS)

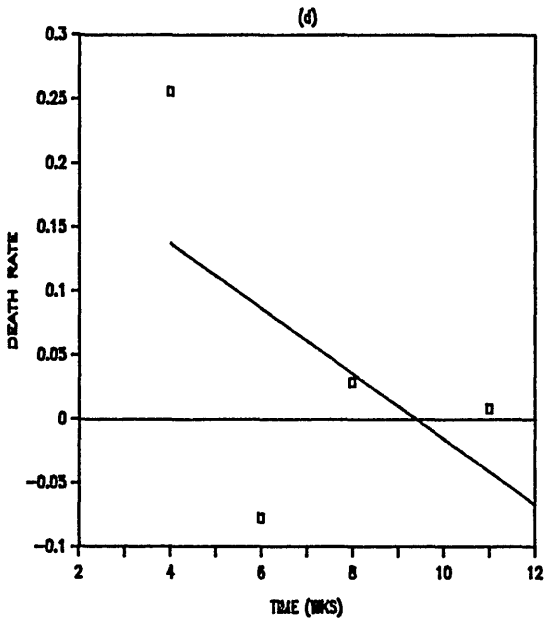

(d)
DEATH RATE
TIME (WKS)

Table 4.4. The average instantaneous rate of mortality, $\bar{\mu}$, of H. polygyrus in outbred MF1, and inbred CBA/Ca and NIH mice.

Mouse strain	Death rate(/worm/week) (± s.e.)	Expected life-span (weeks)
MF1	0.1005 ± 0.0231	9.95
CBA/Ca	0.0279 ± 0.0093	35.8
NIH	0.1115 ± 0.0015	8.9

An increase in the instantaneous death rate/parasite/week with increasing initial dose of infection was apparent (Fig. 4.7) and a single factor analysis of variance revealed a significant difference ($F=3.329$; $0.025<P<0.05$) in the death rate over the range of doses employed in this experiment. The power of the ANOVA for $\nu_1=3$, $\nu_2=15$, $\phi = 1.75$ was estimated at 0.70 (Zar,1984) i.e. there was a 30% chance of having committed a Type II error or not rejecting the H_o (null hypothesis) when the hypothesis was false.

The sex-ratio was female biased, but not consistently so (Table 4.3). A χ^2 test for goodness-of-fit for the pooled data on CBA mice was not significant ($P>0.50$) and the heterogeneity chi-square value was significant ($P<0.001$, D.F.= 22) suggesting that a χ^2 test on the pooled data was not justified.

4.2.3.2 Estimation of parasite reproductive rate

Direct measurement of the intensity of intestinal parasitic infections is usually not possible due to the habitat occupied by the parasite. Indirect measurement of the intensity of parasites has, therefore, relied on, inter alia, the correlation between the size of the parasite population and number of reproductive stages released in host faeces, body fluids or tissues (Stevens,1978; Hall,1981). Such a criterion is, however, fraught with complications, not least of which are the

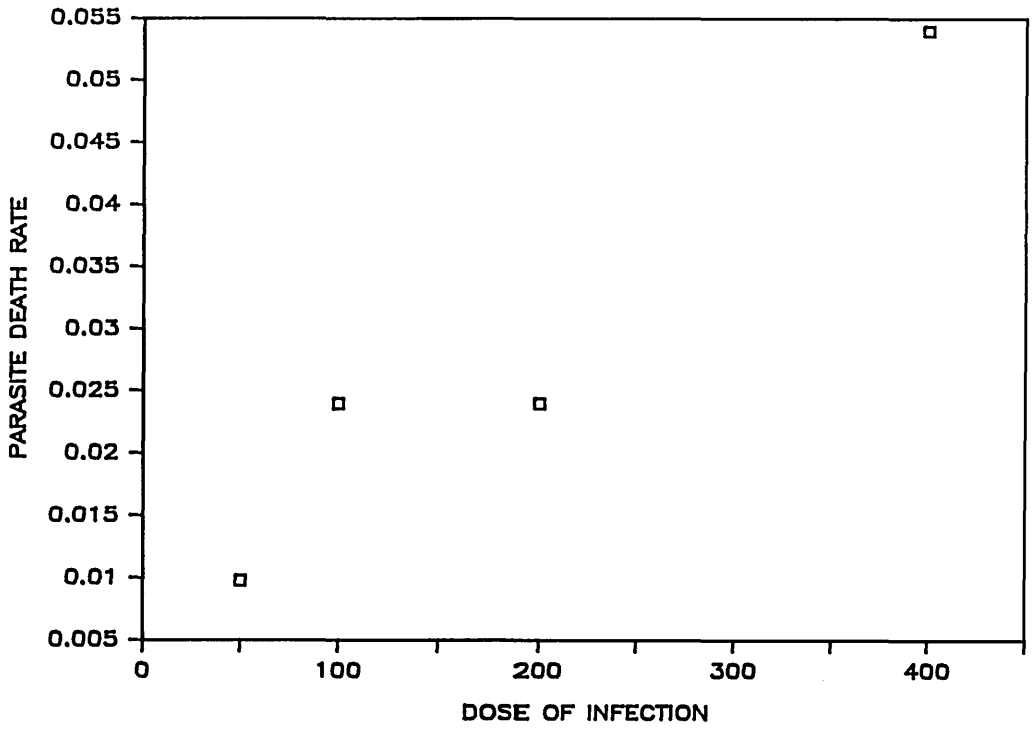


Figure 4.7. The relationship between the instantaneous death rate per parasite per week and the initial dose of infection of CBA/Ca mice with H.polygyrus.

Squares - observed death rate/parasite/week.

Analysis of variance test, $F=3.329$; $P<0.05$.

($F_{0.05}(1)$, 3,15 = 3.29).

errors associated with measurement and those due to the complexity of the biological relationship between host and parasite (MAFF,1977; Hall,1982; Anderson,1978). The observed non-linearity in density-dependence in intestinal helminth fecundity (Anderson,1978; Croll et al,1981; Keymer,1982) and the apparent age-dependent parasite fecundity (Anderson,1976; Kerboeuf,1982) are examples of this complexity. The purpose of the present experiments was to investigate the relationship between parasite density and fecundity in mice infected once with H.polygyrus. An important component of this aim was to assess the accuracy of faecal egg counts as measures of parasite intensity.

4.2.3.2.1 Density-dependent parasite fecundity

Faeces were collected from individual mice from groups of outbred, female and male MF1, and inbred, female CBA/Ca and NIH mice, which had been infected with varying numbers of infective H.polygyrus larvae before post-mortem examination for adult worms (see 4.2.2.1). It was therefore possible to relate the faecal egg production from individual mice to the total number of female worms recovered at post-mortem. Fig. 4.8 shows the results from CBA/Ca mice expressed as the per capita egg production per female H.polygyrus per 16h (Fig. 4.8a) and the per capita egg production per gram of faeces (Fig. 4.8b). The pattern of egg production is clearly

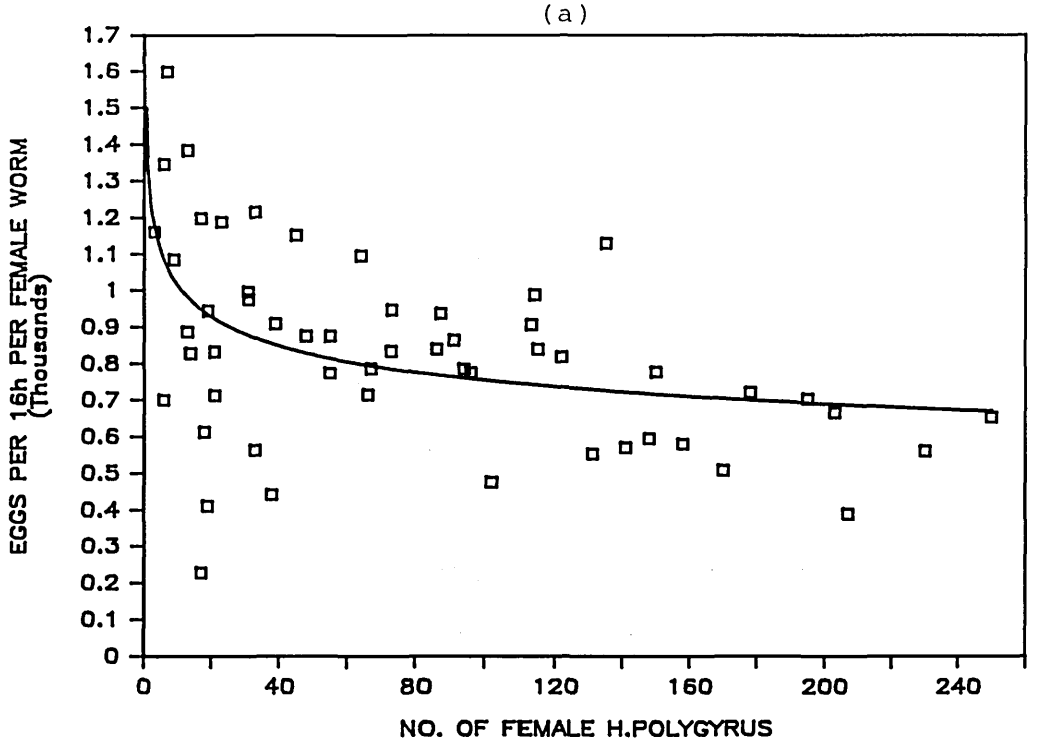
Figure 4.8. Density-dependent per capita egg production by female H.polygyrus in inbred CBA/Ca mice.

Squares - observed fecundity; solid lines - best fit non-linear power models of the form $y = ax^b$.

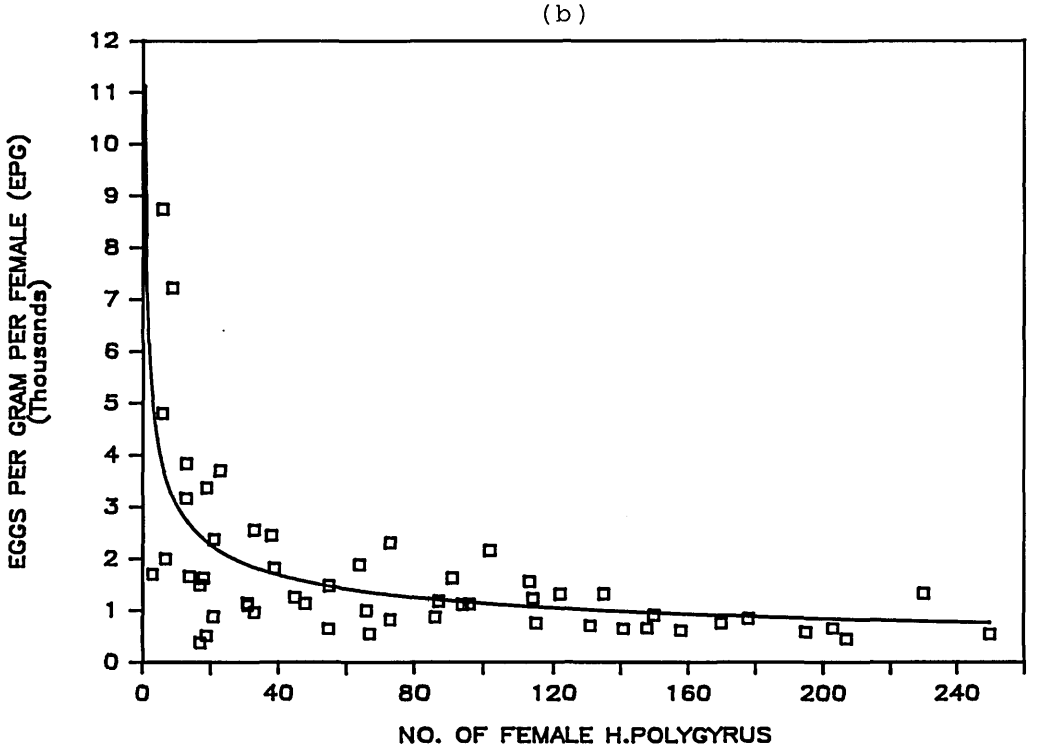
(a) Eggs/female/16h : a = 1369.99; b =-0.129; r^2=0.981;

(b) Eggs/g faeces/female:a = 825.6;b =-0.431; r^2=0.981.

(a)


EGGS PER 16h PER FEMALE WORM (Thousands)
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
40
80
120
160
200
240
NO. OF FEMALE H.POLYGYRUS

(b)


EGGS PER GRAM PER FEMALE (EPG) (Thousands)
12
11
10
9
8
7
6
5
4
3
2
1
0
0
40
80
120
160
200
240
NO. OF FEMALE H.POLYGYRUS

density-dependent within the experimental range of 0-250 female worms. A non-linear (least squares) power model of the form $y = a\ x^b$, where the parameter 'b' denotes the severity of the density-dependent constraints on fecundity, was fitted to the data. Similar patterns were obtained from male and female outbred MF1 and inbred, female NIH mice (Fig. 4.9).

4.2.3.2.2 Age-dependent parasite fecundity

Faeces from individual mice were also collected for parasite egg counts from inbred CBA/Ca mice infected with varying doses of infective H.polygyrus larvae over a period of up to 24 weeks (see 4.2.3.1). The per capita fecundity of H.polygyrus was found to be markedly age-dependent (Fig. 4.10). A non-linear model of the form:

$$y = at.\ \exp(-bt) \qquad [4.4]$$

provided a satisfactory fit to the data. Although purely an empirical model, equation [4.4] adequately describes the pattern of parasite egg production as a function of age. A two-factor analysis of variance test with equal replication on log(10) transformed egg counts (to reduce heteroscedacity in the data) revealed a significant effect on the per capita parasite fecundity (λ) due to the dose of infection, but not the time after infection (Table 4.5).

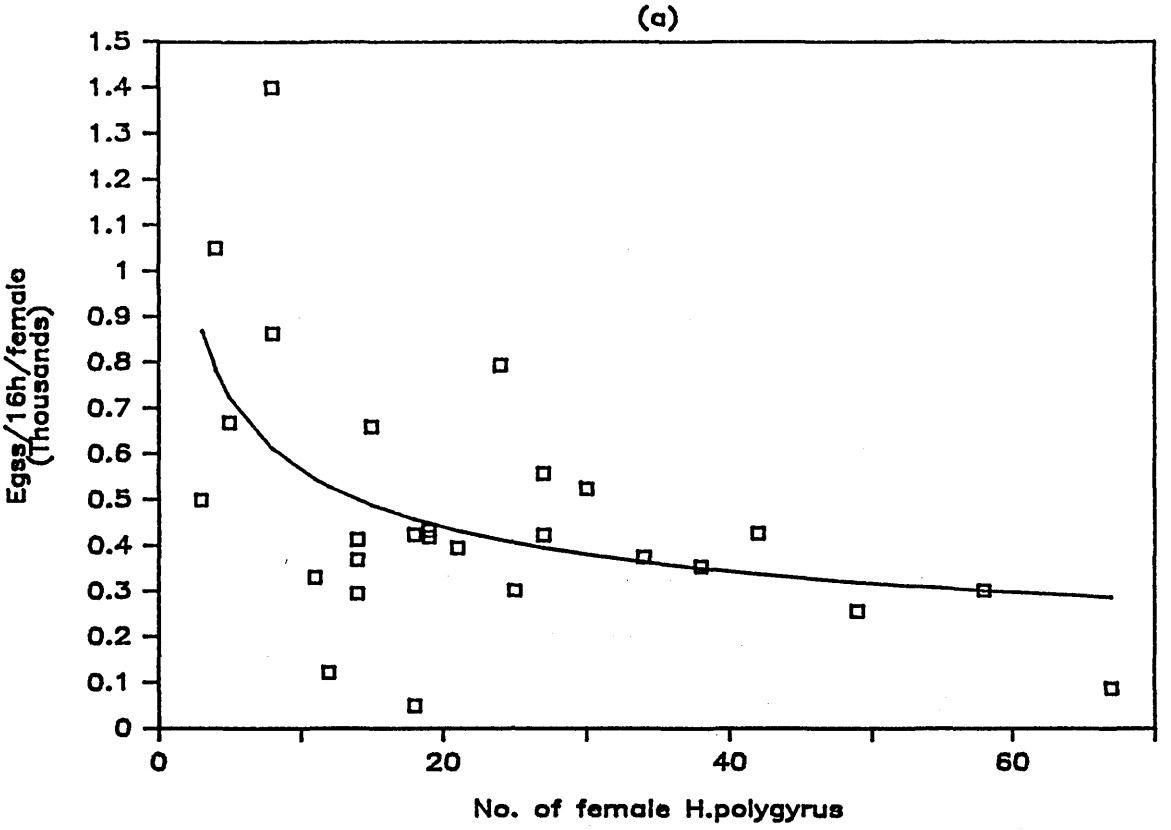
Figure 4.9. Density-dependent fecundity per female H.polygyrus in male (a) and female (b) outbred MF1 mice and in inbred NIH mice (c).

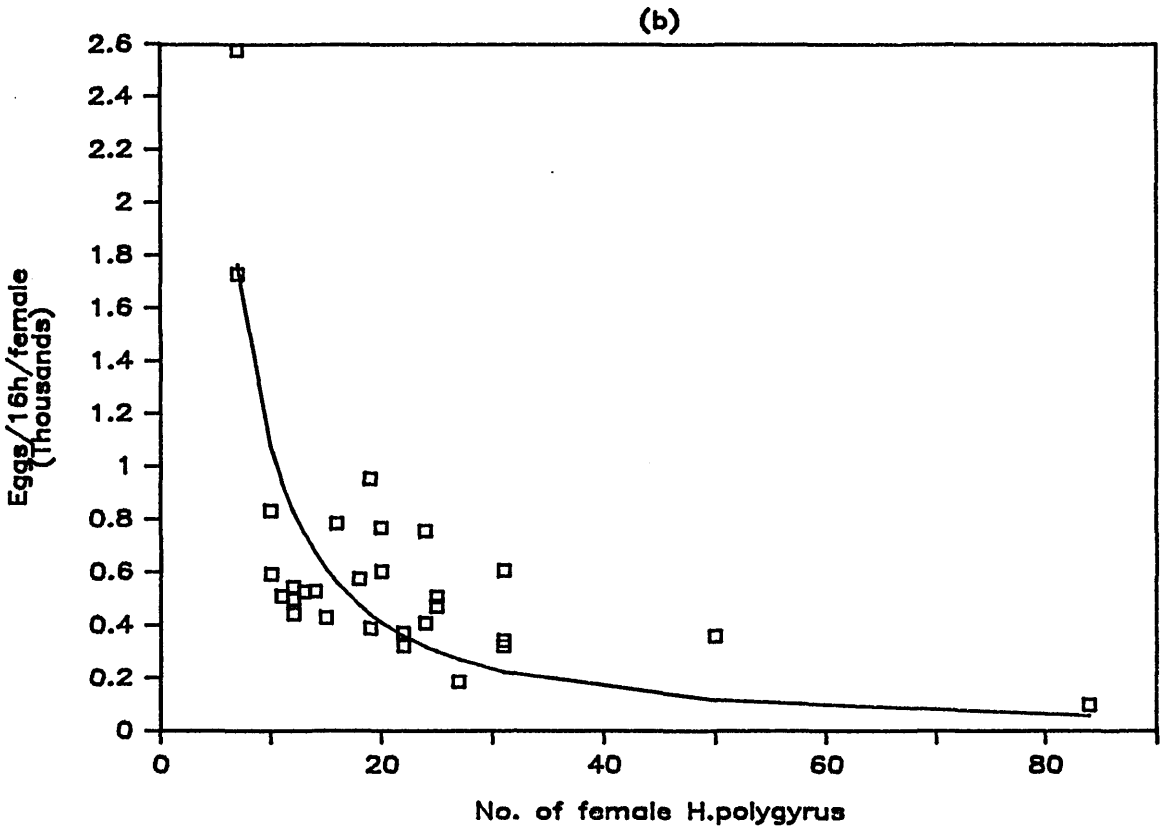
Squares - observed fecundity; solid lines - best fit power models of the form $y = ax^b$, with parameter values:

(a) $a = 1289.52$; $b = -0.3594$; $r^2 = 0.9615$;

(b) $a = 2657.07$; $b = -1.3938$; $r^2 = 0.9643$;

(c) $a = 2583.76$; $b = -1.3585$; $r^2 = 0.9737$.


(a)
Eggs/16h/female (Thousands)
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
No. of female H.polygyrus


(b)
Eggs/16h/female (Thousands)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
No. of female H.polygyrus

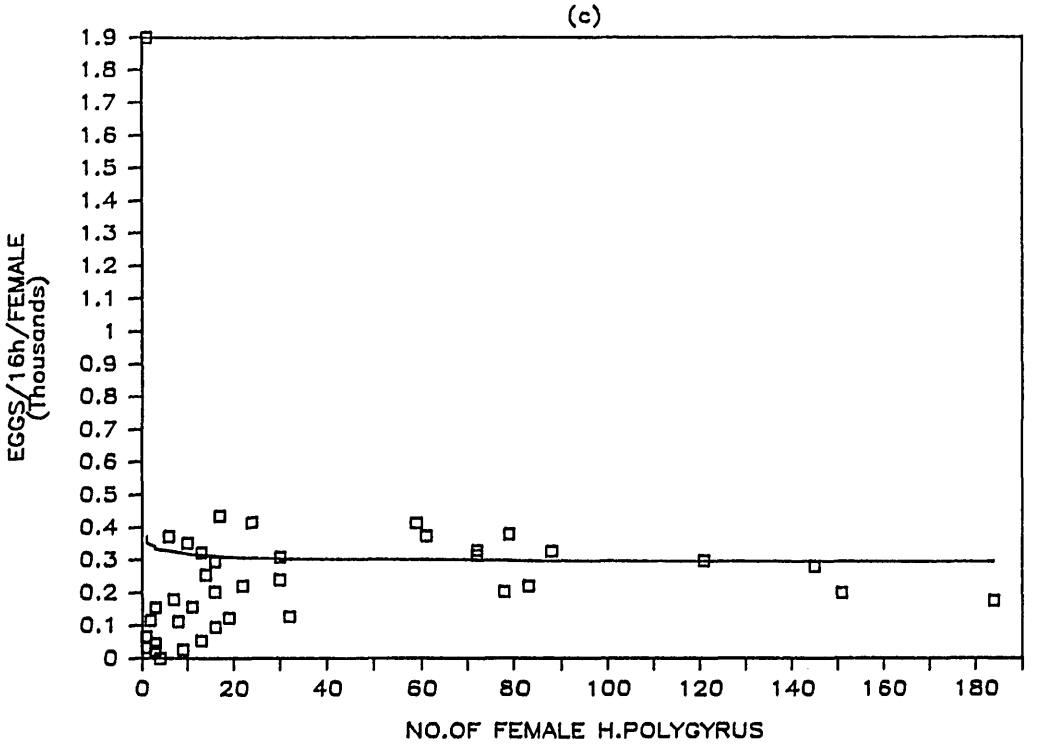

(c)
EGGS/16h/FEMALE
(Thousands)
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
100
120
140
160
180
NO.OF FEMALE H.POLYGYRUS

Figure 4.10. Age- and density-dependent egg production by female H.polygyrus in inbred CBA/Ca mice.

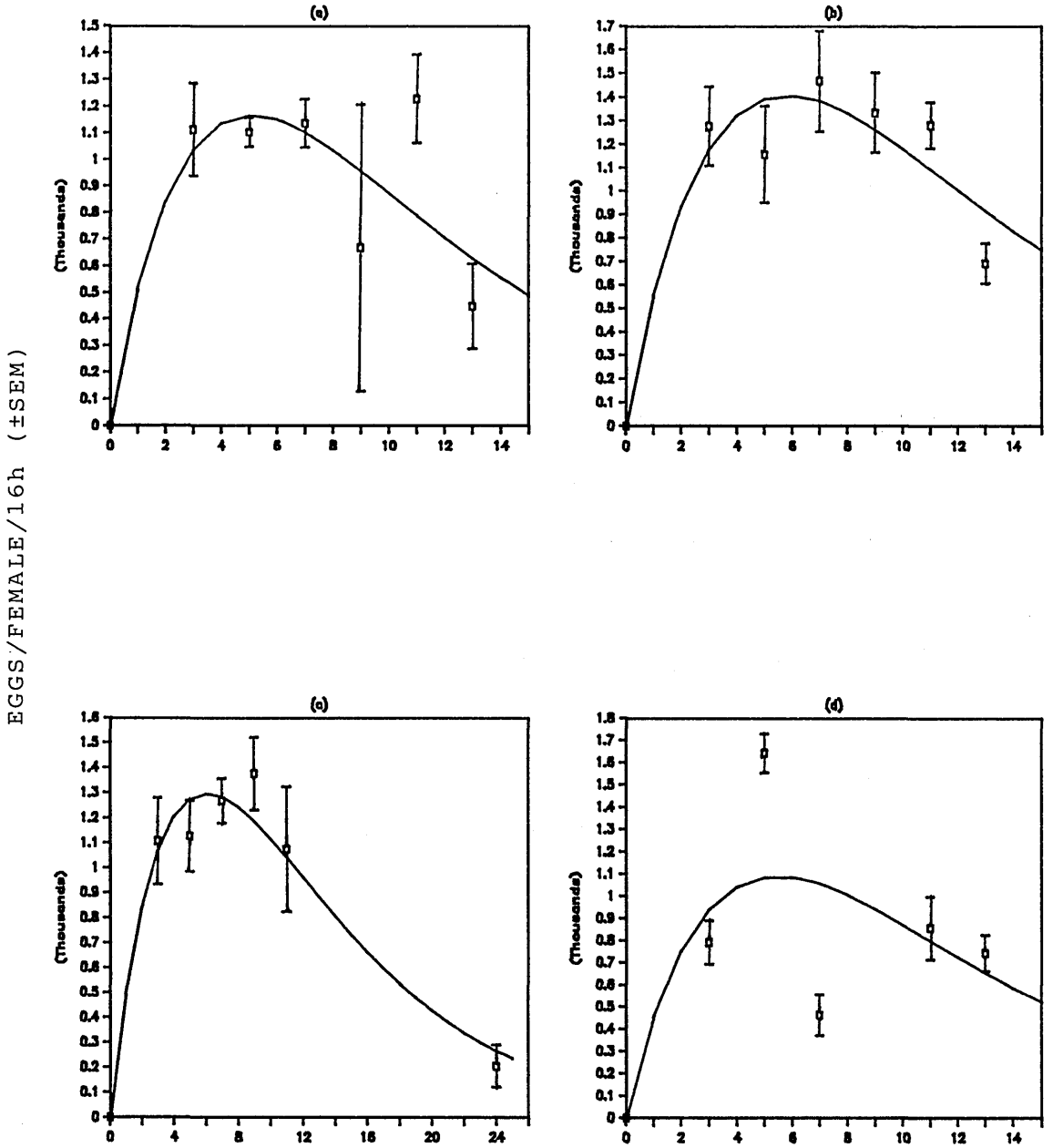
Squares - observed parasite fecundity; vertical bars - the standard error of the mean (n = 6); solid lines - predictions of an empirical non-linear model of the form y = at.exp(-bt); Parameter values (±95% confidence limits):

(a) Dose 50L3 ; a = 624.82 ± 228.0; b = 0.1969±0.064;

(b) Dose 100L3 ; a = 659.43 ± 136.5; b = 0.1723±0.029;

(c) Dose 200L3 ; a = 582.85 ± 75.4; b = 0.1654±0.017;

(d) Dose 400L3 ; a = 543.35 ± 304.6; b = 0.1833±0.117.


(a)
(b)
(c)
(d)
(Thousands)
EGGS/FEMALE/16h (±SEM)
WEEKS AFTER INFECTION

The maximum per capita parasite fecundity (λ_{max}) in CBA/Ca mice can be estimated, by way of an example, from equation [4.4] and λ_{max}, found at time 1/b (≒5 weeks p.i.) was estimated at 1240 ± 70 parasite eggs/16h/female. The total egg production by a female H.polygyrus, T, given an estimated life-span of 35.5 weeks (Table 4.4), was estimated at 18950 ± 1630 eggs from the equation,

$$T = \int_{t_1}^{t_2} at.\ \exp(-bt)\ dt \qquad [4.5]$$

where t_1 is the pre-patent period and t_2 is the expected life-span of the parasite. To obtain the total egg production by a cohort of parasites at time t, (N,t), this term (N,t) is included in equation [4.5] above (Mills, Anderson & Whitfield,1979).

4.2.3.2.3 Results from 'immune' mice

Single infections in naive mice provide a means of estimating the survivorship and fecundity of parasites in the absence of past experience of infection. In nature, the host response to infection e.g. via immunity, has been found to adversely affect both parasite survivorship and fecundity (Wakelin,1985a).

It was of interest, therefore, to investigate the survivorship and fecundity of H.polygyrus in 36 CBA/Ca mice (6-8 week old) immunized by three weekly oral infections of 55 L3 H.polygyrus/mouse. Mice immunized in

Table 4.5. A two-way analysis of variance on log(10) transformed faecal H.polygyrus eggs/16h, with equal replication, to test the influence of dose of infection and time after infection of CBA/Ca mice.

Source of variation	S.S.	d.f.	M.S.	F	P
Total	6.3849	99			
Dose of infection	0.5406	3	0.1802	3.445	<0.025
Weeks after infection	0.0924	4	0.0231	0.442	N.S.
Dose x Weeks	1.5658	12	0.1305	2.495	<0.005
Error	4.1861	80	0.0523		

N.S. = not significant ($P(\alpha) = 0.05$))

this way are known to acquire significant levels of immunity (Van Zandt,1961; Panter,1969; Van Zandt et al, 1973; Behnke & Wakelin,1977). Following immunization, all mice were treated with ivermectin to remove both immature and mature worms. One week later, all mice were challenged with 200 H.polygyrus larvae per mouse. At 2-weekly intervals, 6 mice were chosen at random and killed to determine their worm burdens after assessing their faecal parasite egg counts.

The results in Fig. 4.11(a) show that whereas parasite establishment was only 30% (lower than the rate of establishment of 55% in control mice), once established, worms survived throughout the period of investigation with a small death rate, μ, equal to 0.00075 ± 0.033 (Fig. 4.11a), comparable to the ones reported in primary infection. However, the variance to mean ratio indicated that the distribution of the adult worms was greatly overdispersed. The fecundity of indiviual worms was age-dependent (Fig. 4.11b) but similar to that of worms from naive mice (Fig. 4.10).

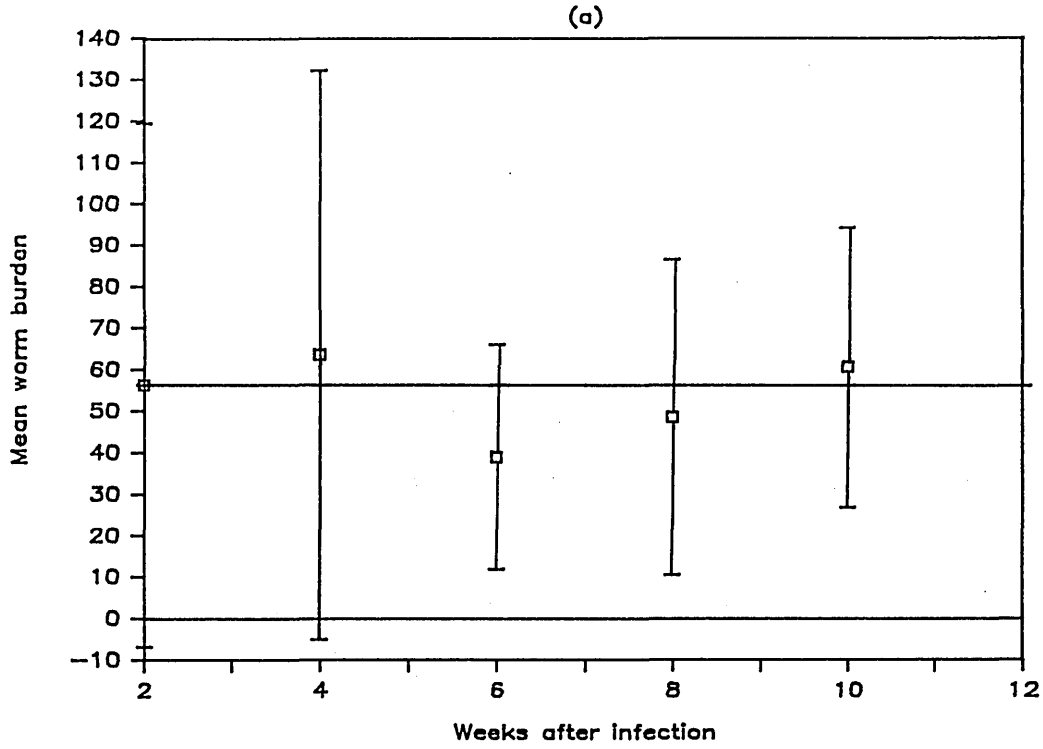
4.3 Results from challenge infections

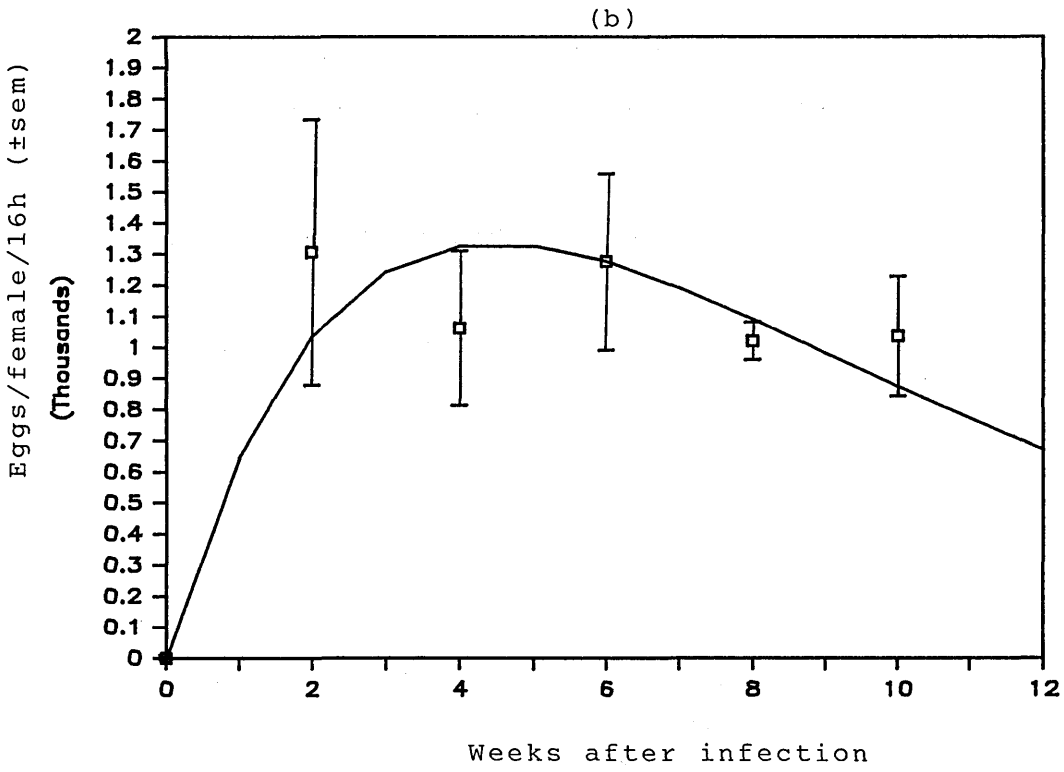
The influence of primary infections on the dynamics of H.polygyrus after challenge infections in mice has been examined by a variety of workers (Dobson, Sitepu & Brindley,1985; Behnke & Wakelin,1977; Behnke & Hannah,1979; Behnke & Robinson,1985; Kerboeuf & Jolivet,1980; Hosier,1974).

Figure 4.11. The mean number of H.polygyrus(a) and the per capita fecundity of female H.polygyrus(b) in inbred CBA/Ca mice immunized by three weekly infections of 55 L3/mouse and then challenged with 200 L3 after chemotherapy.

(a) Squares - observed data (±s.e.); solid line - best fit survivorship model, $N(t)=N(0)\exp(-\mu t)$ (see main text). Parameter values:
Dose = 200L3; ln N(0)= 4.03; μ = 0.00075 ± 0.033.

(b) Squares - observed fecundity (±s.e.); solid line- best fit non-linear empirical model of the form $y = at.\exp(-bt)$. Parameter values (±95% confidence limits):
Dose = 200L3; a = 808.8 ± 249.8; b=-0.2228±0.064.


(a)
Mean worm burden
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
2
4
6
8
10
12
Weeks after infection


(b)
Eggs/female/16h (±sem)
(Thousands)
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
Weeks after infection

In the majority of these studies, however, the level of primary infections was varied whereas the challenge dose, given after removal of the primary infections by chemotherapy, was constant between 100-200 L3 H.polygyrus. Experiments designed to investigate the contribution of varying sizes of primary infection to the subsequent acquisition of challenge infections of varying magnitude in outbred mice were performed. A total of 125 female, 6-8 week old, outbred MF1 mice were used. Primary and challenge infections of 25, 150 and 500 L3, representing low, medium and high doses of infection, respectively, were given to individual mice. Challenge infections were administered 1 week following treatment of mice with pyrantel tartrate to remove 14-day old primary infection worms. Control groups for the infectivity or establishment of both primary and challenge worms in naive mice, and also for drug efficacy, were employed (Fig. 4.12).

The mean number of worms recovered 14 days after primary and challenge control infections in naive mice was about 50% of the original dose and did not differ, at each dose of infection, between the two control groups (Table 4.6). Therefore, it may be concluded that the infectivity of the larvae was not changed during the experiment. The drug was found to be 100% effective against 14-day old worms in all drug treatment groups.

Figure 4.12. Experimental protocol to investigate the influence of primary infection level on the subsequent establishment of challenge infections of outbred MF1 mice with H.polygyrus larvae.

Primary infection (day 0-14)		Challenge infection (day 21 - 35)	k (day 35)
A).	I(25)	Rx | -------- I(25)	k
	I(150)	Rx | -------- I(25)	k
	I(500)	Rx | -------- I(25)	k
B).	I(25)	Rx | -------- I(150)	k
	I(150)	Rx | -------- I(150)	k
	I(500)	Rx | -------- I(150)	k
C).	I(25)	Rx | -------- I(500)	k
	I(150)	Rx | -------- I(500)	k
	I(500)	Rx | -------- I(500)	k

I(x) = number of infective H.polygyrus larvae/mouse

Rx = treatment of mice with pyrantel tartrate. Interval between primary and challenge infection is 1 week.

k = killing of mice for determination of worm burdens.

The mean number of worms recovered 14 days after challenge infections from mice given primary infections was lower than that recovered from control (naive) mice (Table 4.6). The protection conferred to all mice which were given a challenge dose of 25 L3 H.polygyrus was greater than 50% in all groups. However, this protection was not significant between the groups infected previously with low, medium and high primary infections (AOV: F=0.80).

Fewer challenge worms established in mice given a high (500L3) than a low (25L3) primary infection when the challenge was intermediate (150L3). Also, high protection levels were observed in mice given high than low primary infections when challenged with the high dose (500L3). In mice given an intermediate (150L3) primary infection, the overall protection was less for those mice given a high (500L3) than a low (25L3) challenge infection. In all experiments, the distribution of worms was highly aggregated (as indicated by a large variance to mean ratio) after challenge infections. This could indicate heterogeneity in acquisition of resistance to infection. In general, high protection levels were recorded from mice infected with a large primary infection and challenged with a large infection. Although this observation could reflect a high degree of immunity due to large antigenic challenge, severe gastritis (Cypess et al,1974) was evident in mice given high doses of H.polygyrus.

Table 4.6. The mean worm burdens in outbred, female MF1 mice given varying levels of primary and challenge infections of H.polygyrus. Worms were recovered 14 days after infection (see Fig. 4.12 in text).

Primary Infections

Group	n	Dose of infection	MWB ± S.D.
A	5/6	25	13.60 ± 4.0
B	6/6	150	49.67 ± 15.5
C	3/5	500	251.00 ± 49.4

Challenge infections

Group	n	I_P	I_{Ch}	MWB ± S.D. (Control)	n	MWB_{ch} ±S.D.	%Prctn	P*
A	6/6	25	25	14.0 ± 3.2	6/6	3.83±2.7	72.6	0.0002
	6/6	150	25			6.83±5.3	51.2	0.023
	5/6	500	25			5.60±3.8	60.0	0.006
B	6/6	25	150	69.33±14.5	6/6	46.67±41.5	32.7	0.25
	6/6	150	150			29.00±21.3	58.2	0.005
	5/6	500	150			23.20±31.7	65.9	0.03
C	6/6	25	500	238.25±27.3	4/6	233.33±55.1	2.1	0.86
	5/6	150	500			169.00±102	29.1	0.22
	6/6	500	500			25.00±49.9	89.5	0.0001

n = number of surviving mice (out of total)

I_P = primary infection ; I_{Ch} = challenge infection

MWB = mean worm burden (± standard deviation)

Prctn = percentage protection (of challenge)

* = t-test (experimental(MWB_{Ch}) vs challenge control)

Mortality of the infected mice was observed to be higher in those mice given high doses of infection than those given low doses of infection. Parasite-induced host mortality was estimated at 3.78×10^{-4} /parasite/ mouse/unit of time.

4.4 Discussion

Single infection experiments have been described and estimates of the rate parameters that determine the population dynamics of H.polygyrus infection in laboratory mice are provided. The parameters will be useful in the analysis of repeated infection experiments described in chapter 5.

Establishment of H.polygyrus was found to be dose-dependent within the range of doses tested, but the rate of parasite establishment was found to be independent of the strain of the mouse. Density-dependence in parasite survivorship and fecundity was observed. Parasite mortality and fecundity were only adversely affected at very high parasite burdens. The form of density-dependence was such that reduced fecundity was evident at low to moderate parasite burdens but tended to attain a stable level, around 300 eggs/female worm/16h, for worm burdens of 50 or more females (Figs. 4.8 & 4.9).

Density-dependent parasite fecundity is important in the regulation of parasite populations (Anderson,1978).

The precise form of such a regulatory effect will critically depend on the statistical distribution of parasite numbers per host (Anderson & May,1978). Very high parasite burdens per host are unlikely to be encountered frequently in H.polygyrus infections in its natural hosts (Lewis,1968a; Murua,1978). Therefore, the severity of density-dependent constraints in the regulation of natural H.polygyrus infections remains to be demonstrated.

Clearly, the use of egg counts will tend to underestimate parasite burden at low parasite burdens, and overestimate it at high parasite abundance. The need to take account of such variability in the assessment of epidemiological studies of nematode infections (Croll et al, 1981; Hall,1982; Keymer,1982; Anderson & Schad,1985) and Schistosoma mansoni infections in man (Medley & Anderson, 1985) has already been emphasized.

The absence of a marked reduction in parasite fecundity and mortality in mice which were 'immune' (Fig. 4.11) suggests that fecundity was reduced due to parasite senescence, rather than host responses in low responder CBA/Ca mice. Immunity in mice may suppress fecundity of H.polygyrus (Pritchard & Behnke,1985; Sitepu et al,1985).

Acquired resistance in outbred MF1 mice was related to the past experience of a primary infection. In general, high doses of primary infections conferred greater resistance to large rather than small challenge infections. No residual immunosuppression of mice by adult worms was observed.

Chapter 5

Population dynamics of *H.polygyrus* in high and low responder inbred mice

5.1 Introduction

Previous studies of acquired resistance to helminth infections have employed large single, challenge or multiple dose experimental infections (Dineen & Wagland, 1966; Jarrett, Jarrett & Urquhart,1968; Miller,1978; Brindley & Dobson,1983; Dean,1983; Carroll & Grove,1985; Behnke & Robinson,1985). However, it has been suggested that studies that involve the exposure of laboratory hosts to long-term repeated infections have many advantages over the conventional experimental designs (Crombie & Anderson,1985; Anderson & May,1985b). Long-term trickle infections, incorporating the quantitative sampling of the host and parasite populations, provide information on the dynamical changes in parasite establishment and mortality in the hosts. Trickle infection experiments can, therefore, help in the interpretation of observed epidemiological trends in parasitic infections in human and animal communities (Crombie & Anderson,1985).

Circumstantial evidence suggests that in endemic areas, helminth infections such as hookworm, are acquired continually at low levels of exposure over long periods of time (Miller,1979; Schad, Nawalinski & Kochar,1983). Studies of the survival of hookworm larvae using damp pads have revealed a highly overdispersed distribution of larvae at the topmost surface layer of the soil (Hominick, Dean & Schad,1986; Nwosu & Anya,1980).

Adverse environmental factors combined with human avoidance of contaminated soil play an important role in determining the rate of infection (Schad et al,1983). Massive infections are likely to be rare (Schad et al, 1983) but an isolated case of very heavy infection with Ascaris acquired over a short interval of time has been reported (Baird, Mistrey, Pimsler & Connor,1986). The rate of infection of hosts by parasites of veterinary importance (such as the gastrointestinal nematodes of sheep and cattle) increases through time (as the host ages) due to the build up of pasture contaminations via prolonged grazing on a single habitat (Michel,1963; 1970; Kerboeuf & Jolivet,1984; Smith & Grenfell,1985). However, experimental designs in which the infection rate is not held constant are difficult to interpret and analyse in the absence of detailed information on the temporal pattern of exposure.

Experimental trickle infection studies have largely involved mammal host-nematode parasite models. The early experiments of Michel (1963) with Ostertagia ostertagia in calves; Haemonchus contortus in sheep (Dineen & Wagland, 1966; Pradhan & Johnstone,1972) and more recently - employing radiolabelled parasites as tracers in sheep exposed to 600, 1200, 2400 and 4800 H.contortus larvae/sheep/week (Barger, LeJambre, Georgi & Davies,1985; Barger,1986) - indicate the relevance of this approach to an understanding of the dynamics of economically important infections.

Trickle infection experiments have also included the study of Nippostronylus brasiliensis at the rate of 5 and 50 larvae/rat/week day in Sprague-Dawley rats (Jenkins & Phillipson,1971; 1972), Trichuris muris in outbred mice (Wakelin,1973) and the filarial parasite Brugia pahangi in cats (Denham, Ponnudurai, Nelson, Rogers & Gray,1972). Experimental repeated infections have not, however, been confined solely to mammal-parasite systems. Anderson (1976) reported experiments in which groups of the cyprinid fish, Brachydanio rerio, were exposed to Transversotrema patialense at the rate of 2 cercariae/fish/week over a 20-week period.

The mouse hookworm, H.polygyrus in outbred mice has been suggested as a useful model to study the development of acquired resistance in man (Bartlett & Ball,1972). Outbred mice are heterogeneous (Price & O'Brien,1980; Festing,1982) and their use could reflect the expected responses in natural host communities (Wakelin,1982). For purposes of analysing the mechanisms of resistance to helminth infections, however, inbred mice are preferable (Mitchell,1979; Wakelin,1982). Homogeneous responses to infection can be expected from inbred mice (Bailey,1980). Also, genetically-defined mouse strains are widely available (Wakelin,1985b), thus facilitating laboratory experimentation.

Mouse strain variation in the development of resistance to H.polygyrus has been well documented (Liu,1966; Cypess & Zidian,1975; Lueker & Hepler,1975; Behnke & Wakelin,1977; Prowse, Mitchell, Ey & Jenkin, 1979; Behnke & Robinson,1985). Therefore, analysis of the mechanisms of resistance to H.polygyrus must take into account the strain of mouse employed in the experimental design (Prowse & Mitchell,1980) and the types of parameters measured (Table 5.1).

To obtain quantitative information on the role of host genetic background on the dynamics of parasite populations, two inbred mouse strains NIH (haplotype H-2q) and CBA/Ca (H-2k), designated as high and low responders respectively, were chosen for study (see Table 5.2). The experimental approach was to expose cohorts of NIH and CBA/Ca mice to repeated infection with H.polygyrus at three rates of infection held constant through time. The low (10L3/mouse/week), medium (25L3/mouse,week) and high (50L3/mouse/week) doses of infection were chosen to mimic low, medium and high transmission intensities in areas endemic for intestinal parasites. Next, the changes in parasite establishment and mortality wer monitored by sampling the parasite populations at 2-weekly intervals, during a 12-week experimental period.

Table 5.1 Parameters measuring the resistance of mice to infection with H. polygyrus.

A) Innate resistance
   1. Parasite establishment, survival, fecundity
   2 Parasite-induced host mortality
   3. Host sex differences
   4. Host specificity

B) Acquired resistance
   1. Parasite establishment: effect of previous infection, mode of infection, chemotherapy
   2. Parasite survival and expulsion
   3. Host sex differences
   4. Impact of parasite on host growth and survival
   5. Immunological resistance
      a) ratio of encysted larvae to granulomata
      b) immunomodulation of host by adult worms
      c) production of antibody
      d) passive transfer of antibody
      e) immunization with adult worms
      f) adoptive transfer of lymphoid cell and antibody
      g) adoptive transfer of polymorphonuclear cells plus antibody
      h) immunization with worm extracts
      i) cell-mediated responses

Table 5.2 Parameters measuring phenotypic variation in response of inbred mice to H.polygyrus

Mouse strain	Phenotype	H-2 haplotype	Parameter +	Response	Reference*
NIH	high responder	q	(1)	very good	a, b
			(2)	good	c
			(3)	good	d
			(4)	reduced	d
			(5)	good	d
			(6)	average	b
			(9)	very good	d
			(7),(8)	-	N.D.
CBA/Ca	low responder	k	(1)	poor	e,b,f
			(2)	poor	g,h
			(3)	poor	h
			(4)	good	d
			(5)	good	d
			(9)	good	d
			(6),(7), (8)	-	N.D.

\+ 1. Worm burden following challenge
2. Worm burden following multiple infections
3. Host survival and growth
4. Parasite fecundity and growth
5. Antibody production
6. Cell transfer
7. Cell-mediated immunity
8. Cyst-granulomata ratio
9. Vaccination with irradiated larvae

* (a) Behnke & Parish,1979; (b) Behnke & Robinson,1985; (c) Behnke & Wakelin,1977; (d) this study; (e) Prowse *et al*, 1979; (f) Prowse & Mitchell,1980; (g) Mitchell,1979; (h) Mitchell & Prowse,1979; N.D.= not done.

The experiments also provided an opportunity to test predictions based on recent theoretical studies of the factors that generate observed epidemiological patterns in human communities infected with intestinal nematodes (Anderson,1976; Anderson & May,1985a,b). Mathematical models, coupled with empirical data, can facilitate the study of observed changes in population and community dynamics. They can also be employed to generate testable hypotheses and thereby aid in the design of experiments. They can also serve as standards against which observed empirical and natural patterns can be compared (Anderson, 1976; 1986; Pielou,1981).

Much debate surrounds the factors responsible for observed age-related changes in the intensity of parasitic infections in man (Warren,1973; Anderson & May,1985a,b) and the generation of the overdispersed distributions of helminth parasites in natural host communities (Anderson, Whitfield, Dobson & Keymer,1979; Bradley,1974; Crofton, 1971; Anderson & Gordon,1982; Anderson & Medley,1985; Wassom, Dick, Arnason, Strickland & Grundmann,1986). The factors that predispose man to hookworm (Schad & Anderson,1985), Ascaris lumbricoides (Elkins, Elkins & Anderson,1986), Trichuris trichuira (Bundy, Thompson, Cooper, Golden & Anderson,1986) and Schistosoma mansoni (Bensted-Smith, Anderson, Butterworth, Capron, Cordingley, Dalton, Dunne, Karinki, Kimani, Koech, Mugambi, Ouma, Prentice, Richardson, Siongok, Sturrock & Taylor,1986) are unclear at present, but experimental studies implicate

genetic, nutritional and behavioural mechanisms (Wakelin, 1985a; Slater & Keymer,1986b; Anderson et al,1979; Holmes,1973). The significance of the influence of the interaction between these factors and chemotherapy or vaccination, on the dynamics of helminth infections in relation to community control of parasites is not well understood at present (Anderson & May,1985b,c; Anderson & Medley,1985).

This chapter is organised as follows: in section 5.2, results are presented showing the mean worm burdens/mouse and parasite fecundity in both low and high responder mice, and in outbred mice during repeated infection. The influence of the frequency of trickle infection is examined in low responder mice in section 5.2.2. In section 5.3, a pure immigration-death model (Anderson,1976) is employed to describe the experimental results from low responder mice. Various mechanisms can generate the observed patterns of worm burdens in the experiments, and section 5.4 examines the role of differential parasite establishment as a function of the accumulated past experience of infection in the two mouse strains. In section 5.5, a model developed by Anderson & May (1985b), which specifically incorporates the effects of acquired immunity and heterogeneity in response to parasites is employed. The main assumption of the Anderson & May (1985b) model is that parasite establishment in hosts repeatedly exposed to infection is inversely related to the accumulated past experience of infection. This model has as yet only

only been tested on experimental data from S.mansoni in mice (Crombie & Anderson,1985). In section 5.6, the effects of the host immunosuppression via infection by H.polygyrus (Shimp, Crandall & Crandall,1975; Chapman et al,1979a; Behnke et al,1983; Pritchard & Behnke,1985; Sitepu, Dobson & Brindley,1985; Sitepu & Brindley,1985) and increased parasite death during repeated infection, are incorporated into a new model built into the Anderson & May (1985b) framework.

In order to gain more information on the influence of host genetic background on the community control of infections, the experiments were extended to include the investigation of the impact of chemotherapy (section 5.7) and vaccination (section 5.8) on the dynamics of trickle infections in both low and high responder mice. The use of chemotherapy during trickle infections permitted the assessment of the duration of acquired resistance in mice (section 5.9). 'Immunological memory' to helminth infections is little understood at present (Bullick, Russell & Castro,1984; Wakelin, Grencis & Donachie,1982; Palmas, Bortoletti, Conchedda & Gabriele,1986; Crombie & Anderson,1985). It is widely believed to be short-lived in man (Cohen & Warren,1982; Barriga,1980; Lloyd,1981; Wakelin,1984). Finally, the results are discussed in relation to the population dynamics of intestinal helminth infections and the prospects for their control.

5.2 Results from trickle infections

5.2.1 Results from inbred low (CBA) and high (NIH) responder mouse strains

One hundred and eighty (180), 6-8 week old CBA/Ca female mice were divided into three groups of 60 mice each. Group I mice were given a low dose of infection of 10 H.polygyrus larvae/mouse/week for 12 weeks. Groups II and III mice were each exposed to medium and high doses of infection corresponding to 25 L3/mouse/week and 50 L3/mouse/week, respectively. At two-weekly intervals, 10 mice from each group were chosen at random and killed to assess their worm burdens after determination of faecal parasite egg counts and collection of blood serum from each individual mouse. At 2-weekly intervals, five naive, age-matched CBA/Ca mice were infected at the same time as the experimental mice to assess the infectivity of the larvae (i.e. 30 mice were used as infectivity controls). The experiment was repeated using one hundred and fifty (150), 6-8 week old, female NIH mice. The mice were divided into three groups of 50 mice each and infected as for CBA/Ca mice. Thirty naive, age-matched NIH mice were employed to check the infectivity of the larvae employed in the experiment.

5.2.1.1 Mean worm burdens

The mean number of worms/mouse recovered from these two mouse strains are shown in Fig. 5.1. The low responder mice accumulated the number of parasites in a manner related to the intensity of infection for the first 6 weeks of trickle infection (Fig. 5.1a). However, from 6 weeks after initial infection to week 12, the worm burdens in CBA/Ca mice exposed to low and medium doses of infection did not increase linearly. In mice exposed to the high dose of infection, worm burdens continued to increase. By 10 to 12 weeks of infection, there was a host mortality of 20 and 30% at the highest dose of infection whereas all mice subject to the low and medium doses of infection survived.

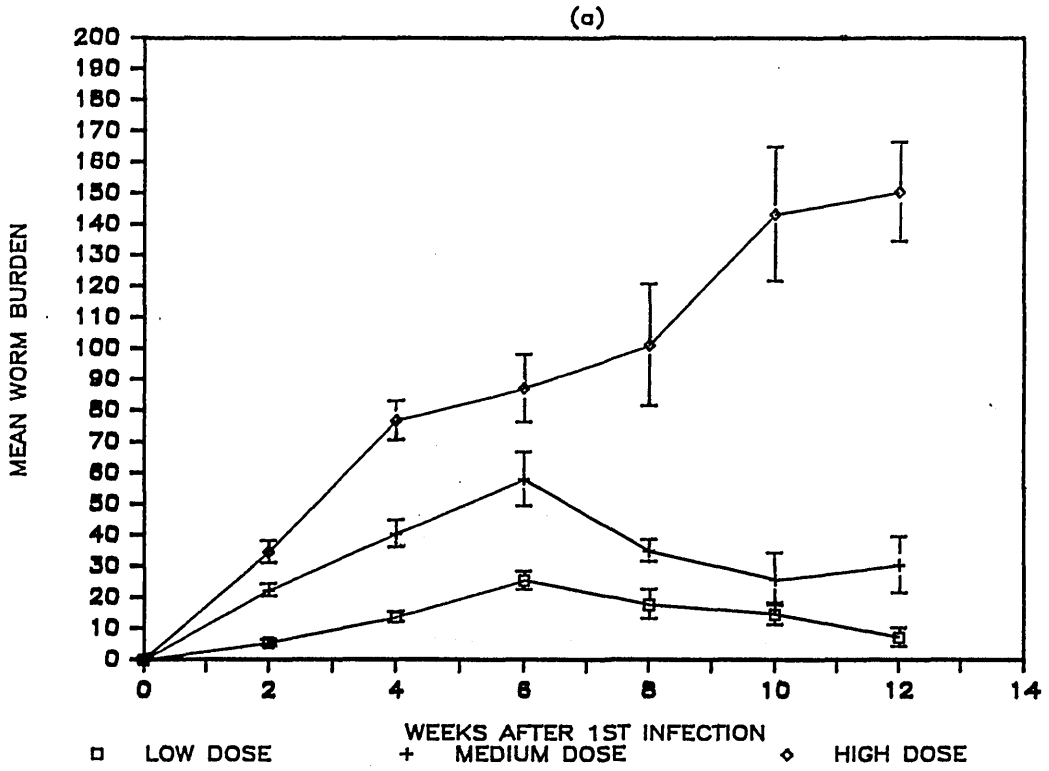
In high responder NIH mice (Fig. 5.1b), the increase in the mean worm burdens was also related to the intensity of exposure to infection. The worm burden/mouse rose to a plateau in the low dose but declined by week 12 after first exposure. At the highest dose of infection, a peak intensity in mean worm burden was attained as early as 2 weeks after the first infection and the parasite burden declined rapidly thereafter. All mice survived during the experiment.

The distribution of H.polygyrus per mouse in both inbred high and low responder hosts became increasingly aggregated as the experiment progressed (Fig. 5.2).

Figure 5.1. The mean number of parasites recovered from low and high responder inbred mice during repeated infection with H.polygyrus.

(a) Observed mean worm burdens (±S.E.) in low responder CBA/Ca mice (10 mice per group) exposed to a low (squares), medium (crosses) and high (diamonds) doses of infection corresponding to 10, 25 and 50L3 per mouse per week.

(b) As for (a), but worms recovered from high responder NIH mice. Note that graphs (a) and (b) are plotted on the same scale for ease of comparison.


(a)
MEAN WORM BURDEN
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
WEEKS AFTER 1ST INFECTION
LOW DOSE
MEDIUM DOSE
HIGH DOSE

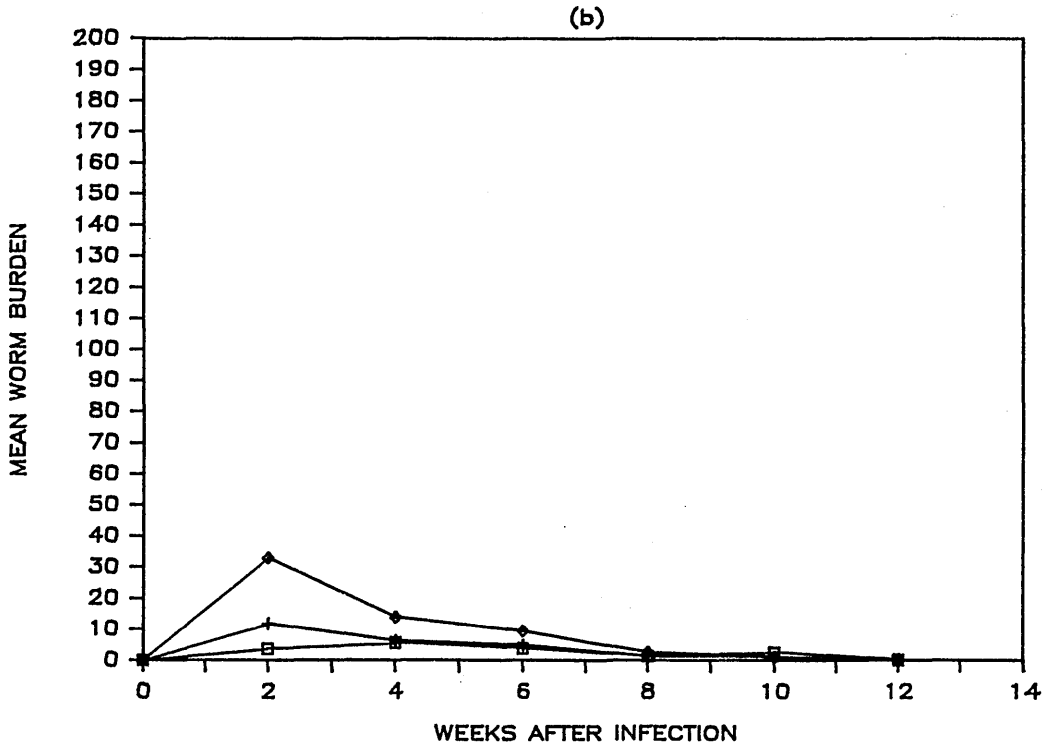
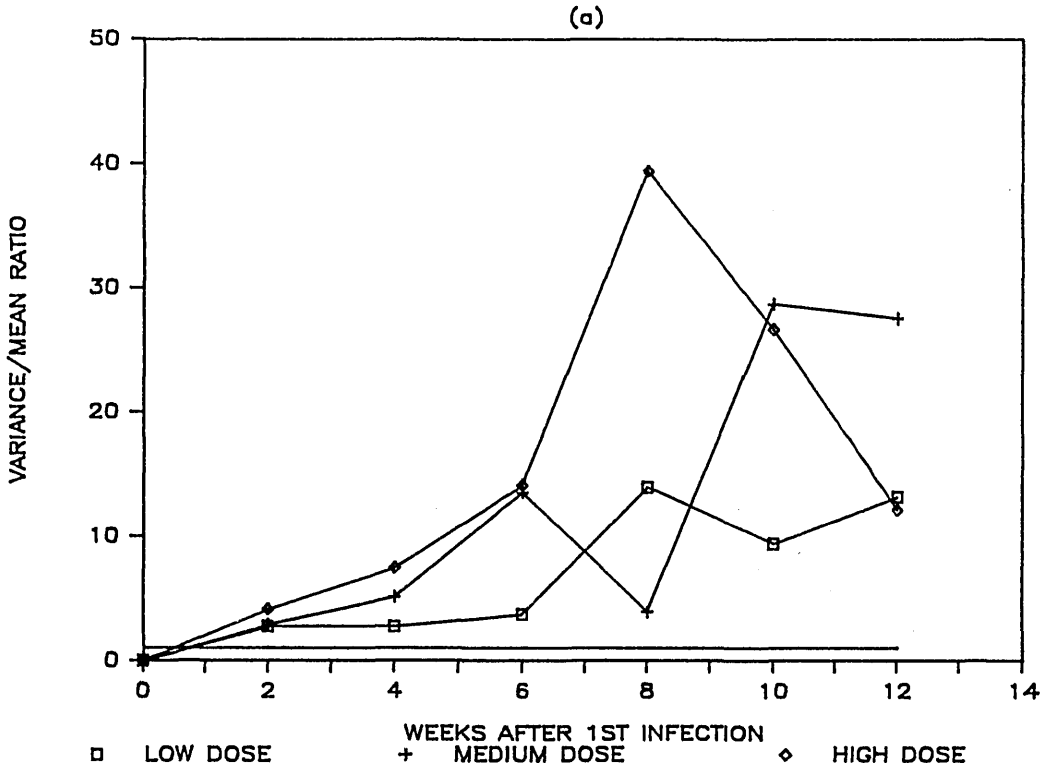
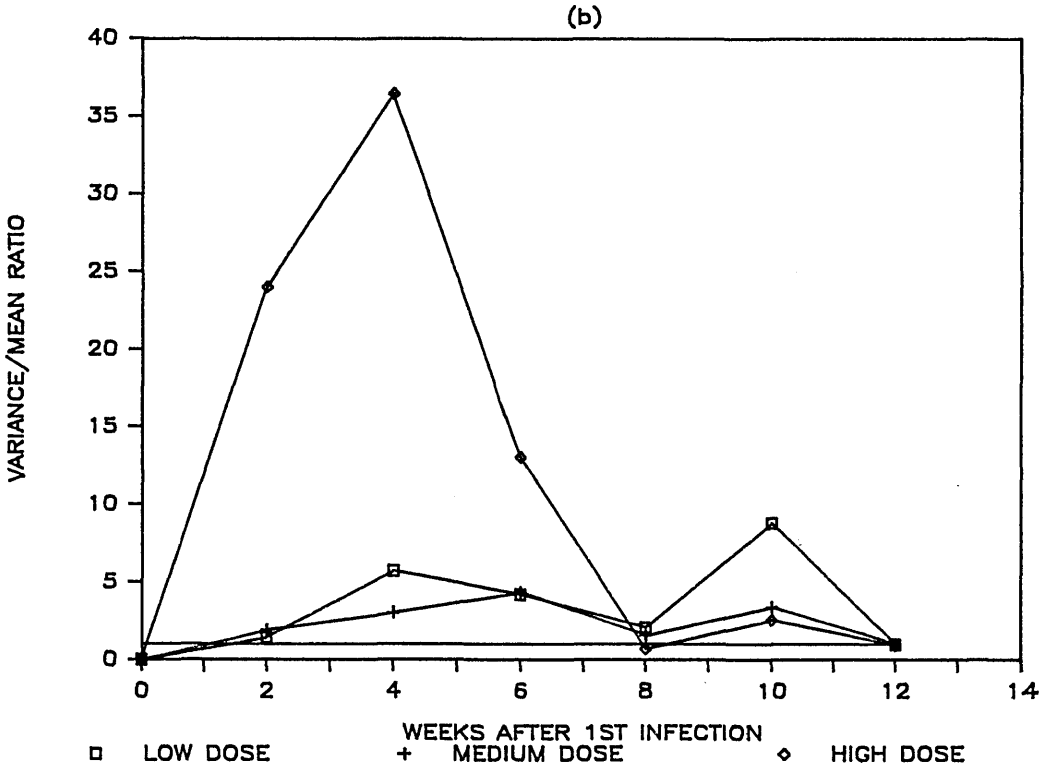

(b)
MEAN WORM BURDEN
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
WEEKS AFTER INFECTION

Figure 5.2. The changes in the variance to mean ratio of the number of H.polygyrus in low (a) and high (b) responder inbred mice during trickle infection.

Groups of 8-10 mice were exposed to 10 (squares), 25 (crosses) and 50 (diamonds) L3 per mouse per week. The horizontal line denotes a Poisson probability distribution where the variance to mean ratio is equal to 1.


(a)
VARIANCE/MEAN RATIO
50
40
30
20
10
0
0
2
4
6
8
10
12
14
WEEKS AFTER 1ST INFECTION
LOW DOSE
MEDIUM DOSE
HIGH DOSE


(b)
VARIANCE/MEAN RATIO
40
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
WEEKS AFTER 1ST INFECTION
LOW DOSE
MEDIUM DOSE
HIGH DOSE

5.2.1.2 Prevalence of infection

The prevalence or proportion of mice infected was always high (100%) in the low responder mice irrespective of the dose of infection (Fig. 5.3a). In high responder mice, the proportion of mice harbouring the infection (measured as the presence of worms) decreased to about 10% by the 12th week of infection (Fig. 5.3b). This suggests that 90% of high responder NIH mice became refractory to infection despite continued exposure.

5.2.1.3 Parasite sex-ratios

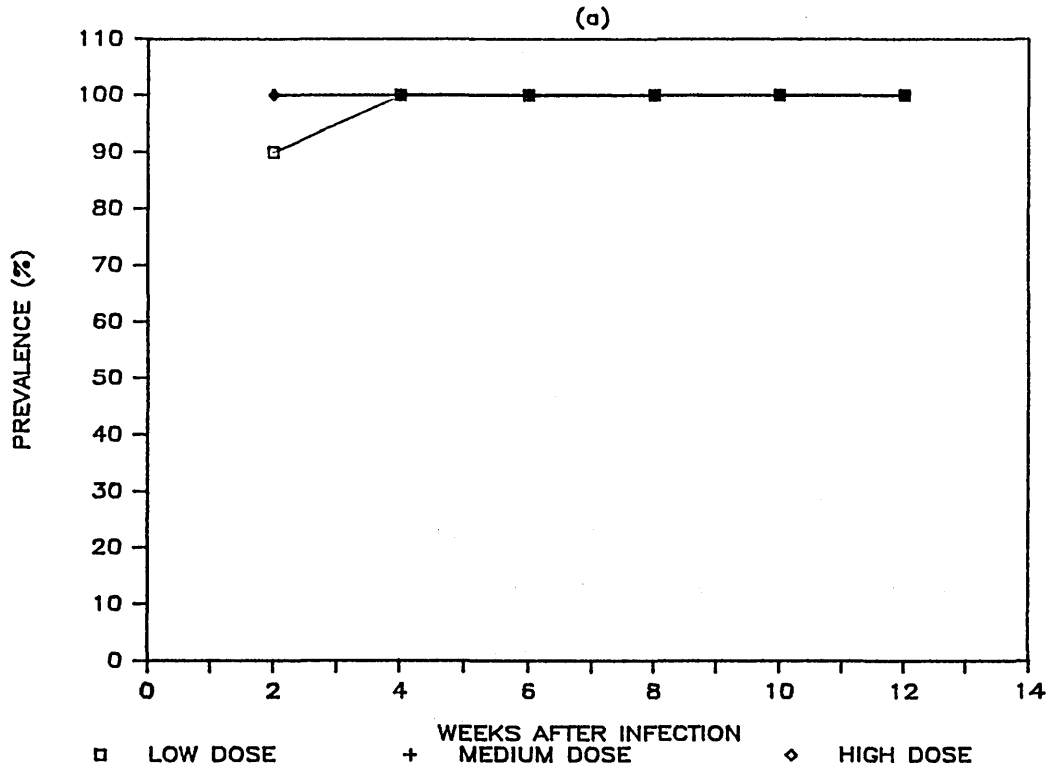
The sex-ratios of the worms in the two mouse strains changed significantly from a 1:1 ratio during the latter part of the experimental period (Fig. 5.4). More female than male worms prevailed towards the end of the experiment (i.e. 8-12 weeks).

5.2.1.4 Parasite fecundity

The per capita fecundity of H.polygyrus in low responder mice was stable at a level of about 600 eggs per female worm per 16h during the course of the trickle infection (Fig. 5.5a). Since worm fecundity is age-dependent during single infections (chapter 4), the observation of a stable egg production suggests that there was a 'turnover' of worms and/or acquired resistance in low responder mice did not have a substantial effect on parasite fecundity.

Figure 5.3. Changes in the proportion of mice infected (% prevalence) with time in low (a) and high (b) responder mice exposed to repeated infection with H.polygyrus.

Groups of 8-10 mice were exposed to infection at the low - 10L3/mouse/week (squares), medium - 25L3/mouse/week (crosses) and high - 50L3/mouse/week (diamonds) doses of infection.


(a)
PREVALENCE (%)
110
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
WEEKS AFTER INFECTION
LOW DOSE
MEDIUM DOSE
HIGH DOSE

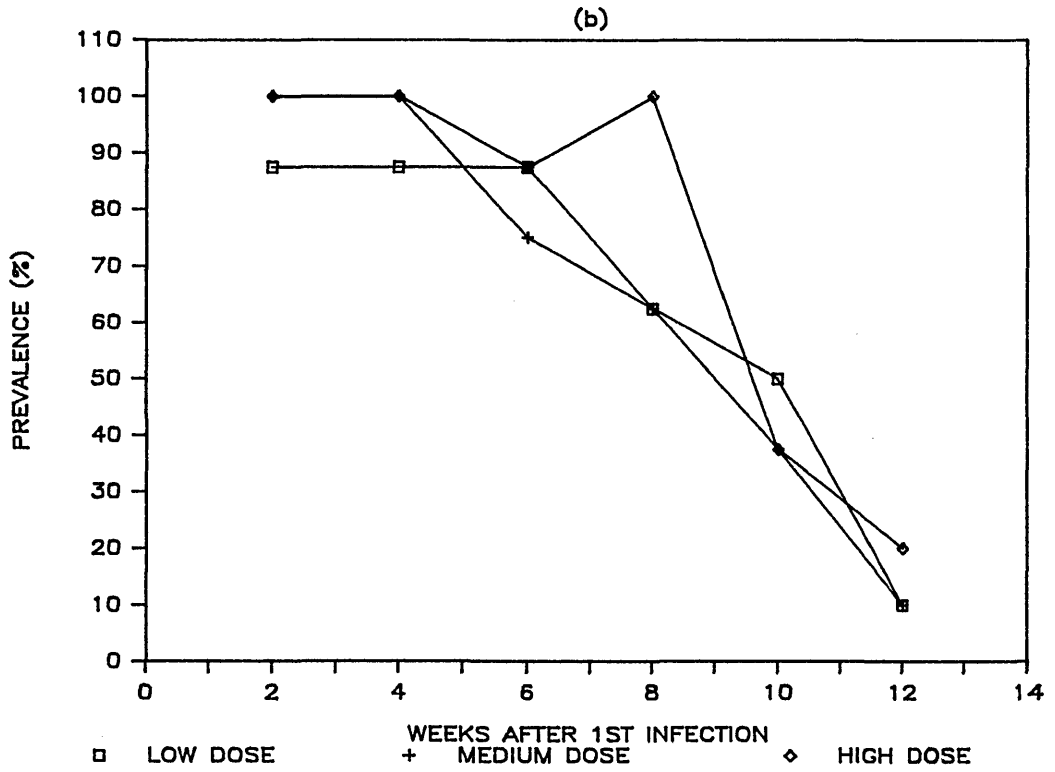
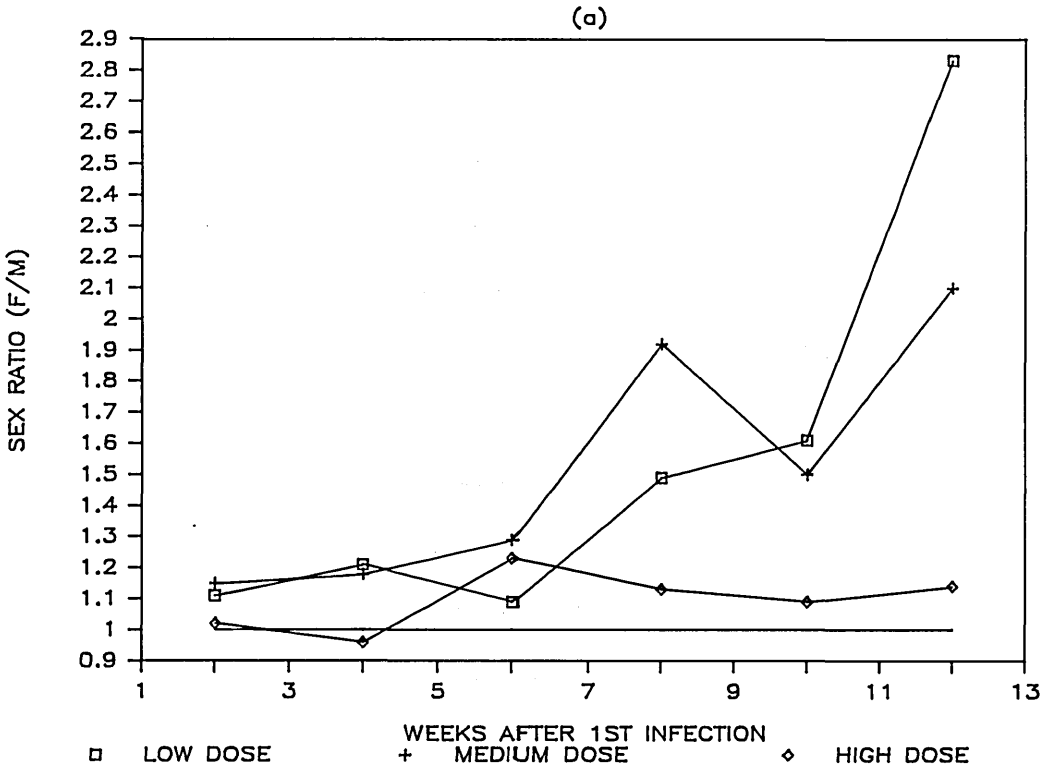
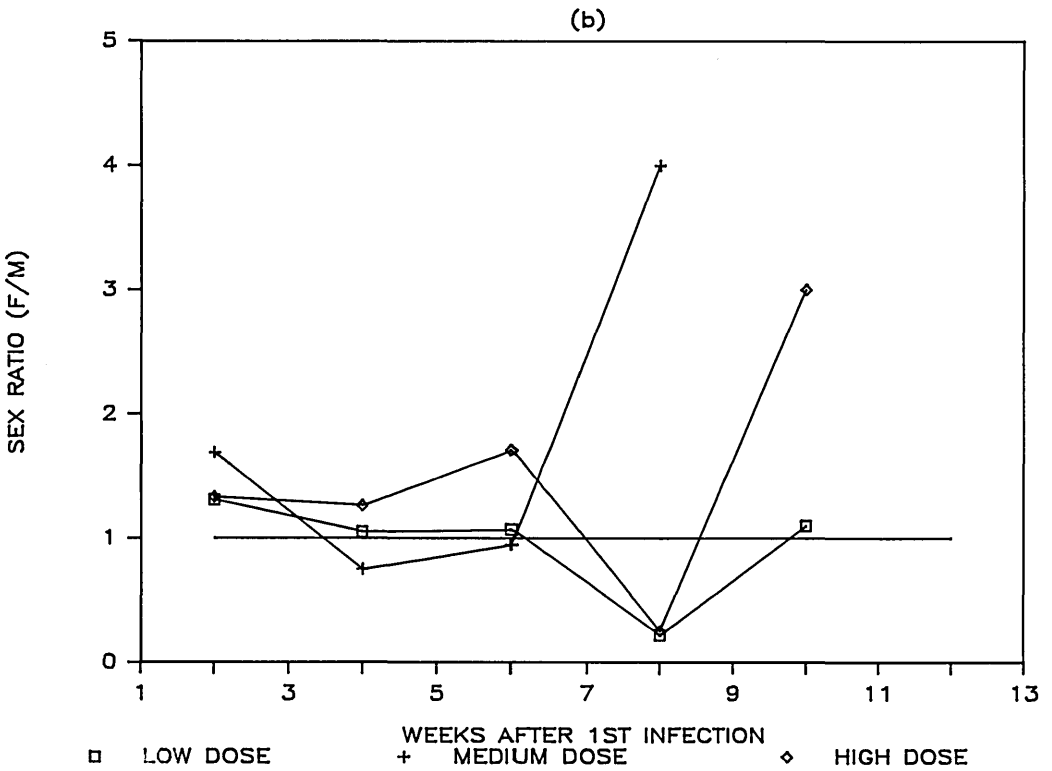

(b)
PREVALENCE (%)
110
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
WEEKS AFTER 1ST INFECTION
LOW DOSE
MEDIUM DOSE
HIGH DOSE

Figure 5.4. Changes in the sex-ratio (female/male) of H.polygyrus in low (a) and high (b) responder mice repeatedly exposed to infection.

Groups of 8-10 mice were exposed to low (squares), medium (crosses) and high (diamonds) doses of infection corresponding to 10, 25 and 50 L3 per mouse per week, respectively. The horizontal line denotes a female to male ratio of 1:1.


(a)
SEX RATIO (F/M)
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
1
3
5
7
9
11
13
WEEKS AFTER 1ST INFECTION
LOW DOSE
MEDIUM DOSE
HIGH DOSE


(b)
SEX RATIO (F/M)
5
4
3
2
1
0
1
3
5
7
9
11
13
WEEKS AFTER 1ST INFECTION
LOW DOSE
MEDIUM DOSE
HIGH DOSE

In high responder mice, the per capita fecundity of H.polygyrus was severely reduced to a lower peak of about 150 eggs/female/16h (Fig. 5.5b). It can be inferred, therefore, that acquired resistance had a great effect on worm fecundity in NIH mice.

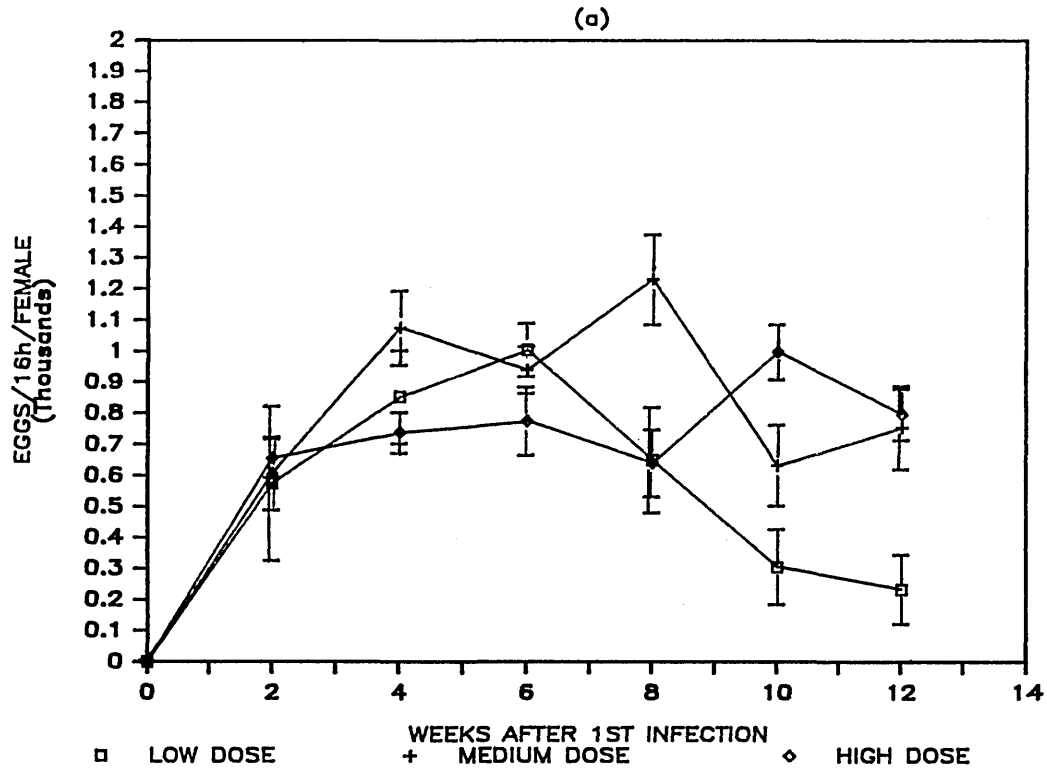
5.2.2 The influence of the frequency of exposure

The influence of the frequency of repeated exposure on the dynamics of the parasite population was examined by infecting low responder CBA/Ca mice with 50 H.polygyrus larvae per mouse per 2 weeks. Forty-eight (48) female CBA/Ca mice were infected and 6-8 mice were killed at 2-weekly intervals during the experimental period of 16 weeks. Twenty (20), sex and age-matched naive CBA/Ca mice were used to assess the infectivity of the larvae.

The results in Fig. 5.6(a) show that the CBA/Ca mice accumulated parasites during the experimental period. The mean worm burden appeared to reach a plateau of about 70 worms/mouse from week 8-16. Care must be taken in the interpretation of this trend because of the high degree of variability seen within the individual mice (Fig. 5.6b). The overall pattern is, however, different from the one displayed in Fig. 5.1(a) for CBA/Ca mice exposed to a more frequent high dose of 50 L3/mouse/week.

Figure 5.5. Changes in the per capita fecundity of H.polygyrus in low (a) and high (b) responder inbred mice repeatedly exposed to infection.

Groups of 8-10 mice were exposed to low (squares), medium (crosses) and high (diamonds) doses of infection corresponding to 10, 25 and 50 L3 per mouse per week. Vertical bars represent one standard error of the mean (SEM).


(a)
EGGS/16h/FEMALE (Thousands)
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
WEEKS AFTER 1ST INFECTION
LOW DOSE
MEDIUM DOSE
HIGH DOSE

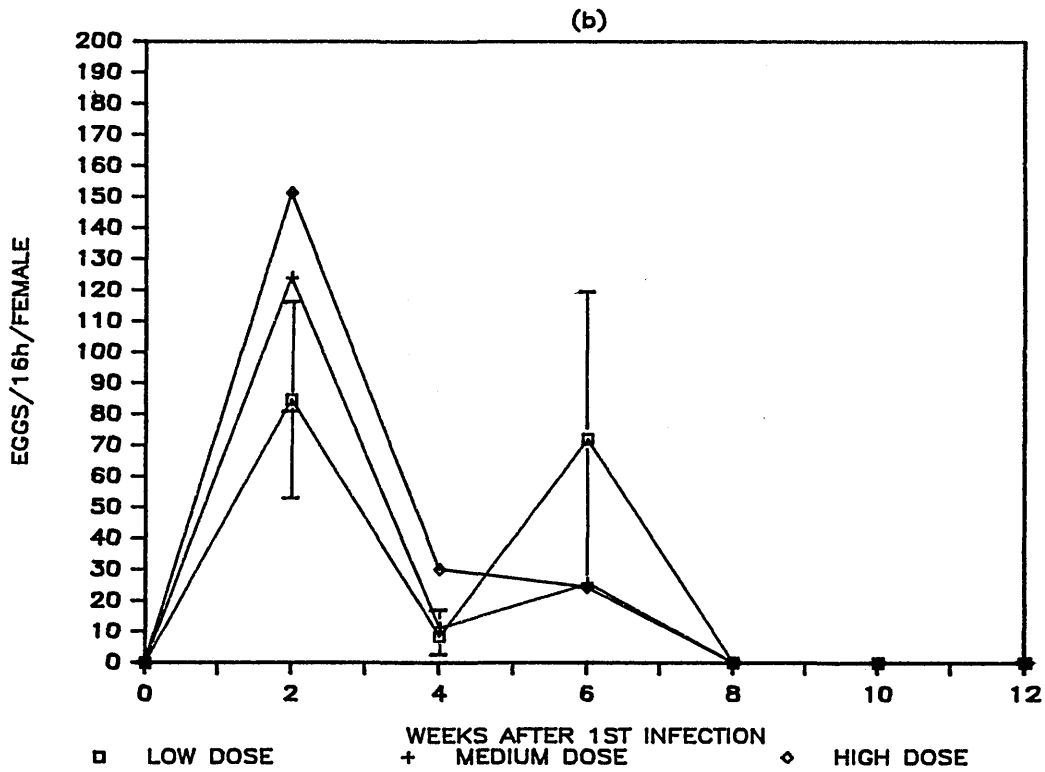
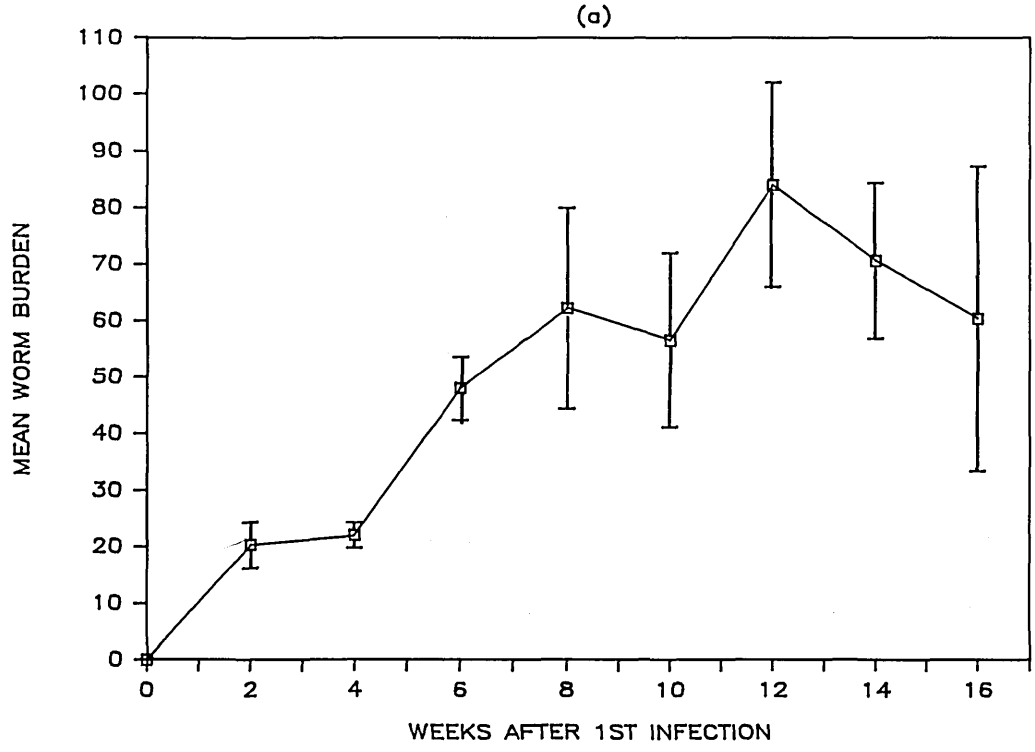
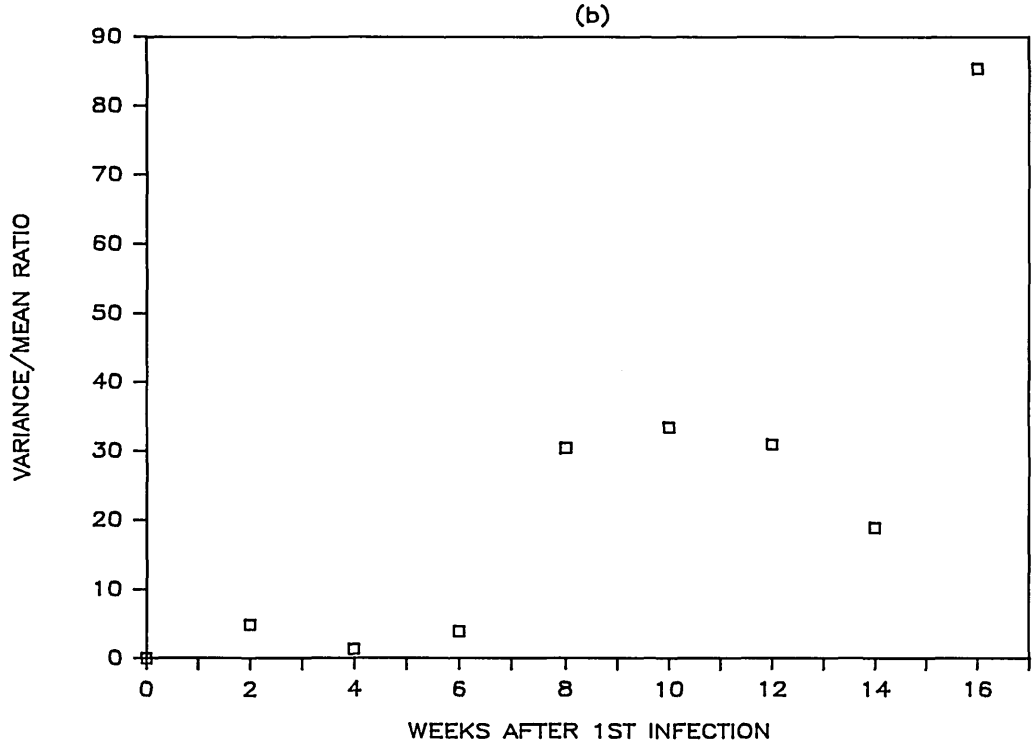

(b)
EGGS/16h/FEMALE
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
WEEKS AFTER 1ST INFECTION
LOW DOSE
MEDIUM DOSE
HIGH DOSE

Figure 5.6. The dynamics of repeated infection of low responder inbred CBA/Ca mice exposed to a high dose of 50 L3 H.polygyrus per mouse per 2 weeks.

(a) Squares - observed mean worm burdens (6-8 mice per group); vertical bars - standard error of the mean.

(b) Squares - the changes in the variance to mean ratio of the mean number of worms/mouse with time post-initial infection.


(a)
MEAN WORM BURDEN
0
10
20
30
40
50
60
70
80
90
100
110
0
2
4
6
8
10
12
14
16
WEEKS AFTER 1ST INFECTION


(b)
VARIANCE/MEAN RATIO
0
10
20
30
40
50
60
70
80
90
0
2
4
6
8
10
12
14
16
WEEKS AFTER 1ST INFECTION

5.2.3 Results from outbred MF1 mice

Forty-eight (48) male, outbred MF1 mice were exposed to a trickle infection of 50 L3 H.polygyrus/mouse/week for 6 weeks and their mean worm burdens were determined thereafter, at weekly intervals. The observed mean worm burden/mouse rose to a peak at 4 weeks after the first exposure and then declined by 6 weeks of infection (Fig. 5.7a). The variance to mean ratio was high during the trickle infection (Fig. 5.7b), indicating an increasing variability among mice in the number of parasites they harboured.

5.3 The immigration-death model

It has been shown that for parasitic species that do not multiply within the definitive host, the number of parasites/host is simply a funtion of the rate at which parasites enter (=immigration rate) and the death rate of the parasite (Anderson,1976). In the absence of density-dependent factors or host immune responses, a simple immigration-death model describes the rate of change of M(t), the number of parasites at time t :

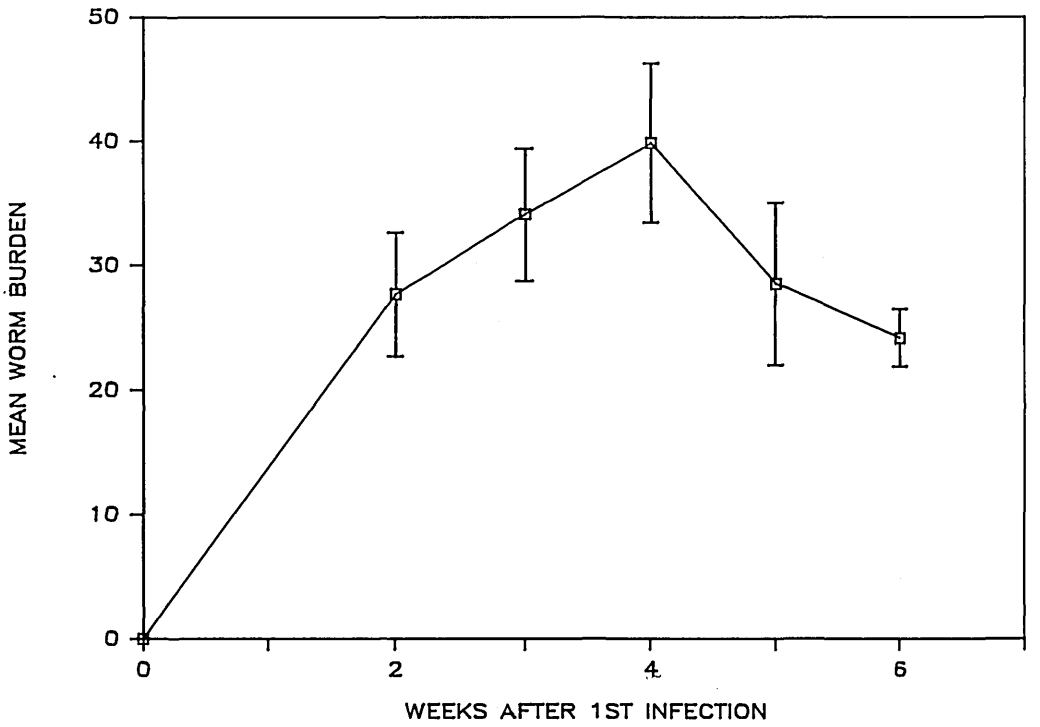
$$dM(t)/dt = \Lambda - \mu M(t) \qquad [5.1]$$

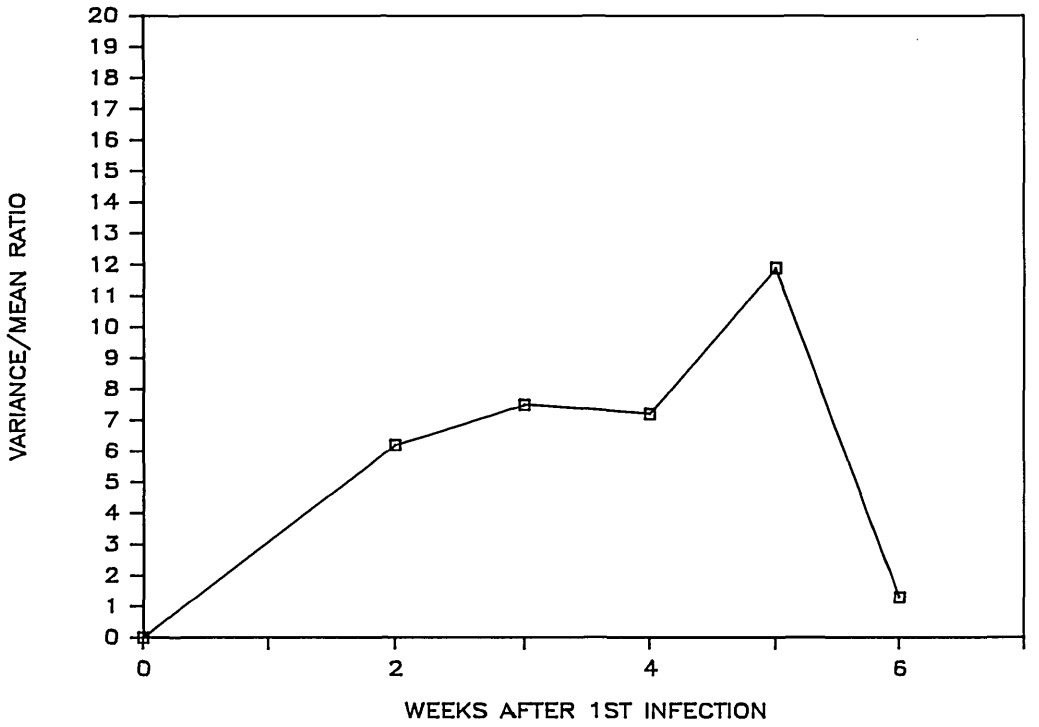
Here Λ is the immigration or infection rate/host/unit of time and μ is the death rate/parasite/unit of time.

Figure 5.7. The dynamics of repeated infection of outbred MF1 mice with a high dose of 50 L3 H.polygyrus/mouse/week.

(a) Squares - observed mean worm burdens (8 mice per group); vertical bars - the standard error of the mean.

(b) Changes in the variance to mean ratio of the number of H.polygyrus per mouse with time.


MEAN WORM BURDEN
50
40
30
20
10
0
0
2
4
6
WEEKS AFTER 1ST INFECTION


VARIANCE/MEAN RATIO
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0
2
4
6
WEEKS AFTER 1ST INFECTION

Given the initial condition M(0) = 0, where M(0) is the number of parasites within the host at time t = 0, then the solution of equation [5.1] is :

$$M(t) = M(0)\exp(-\mu t) + \Lambda/\mu[1 - \exp(-\mu t)] \quad [5.2]$$

This model (eqn 5.1), predicts that the parasite population will grow monotonically to attain an equilibrium size M* where

$$M^* = \Lambda/\mu \quad [5.3]$$

Thus, there exists a steady state which is simply a function of the parameters Λ and μ.

The value of Λ (or Λ_i to denote a particular experiment) is determined by the experimental conditions where the <u>net</u> rate of infection is given by the relationship between λ (the rate of exposure to larvae - set by the experiment) and the rate of parasite establishment b where

$$\Lambda_i = b\lambda_i \quad [5.4]$$

and λ_i defines the experimental infection rate and $i = 1, 2, 3$ (i.e. λ_1 = 10L3/mouse/week; λ_2 = 25L3/mouse/week; λ_3 = 50L3/mouse/week) and the parameter, b , is the slope of the linear relationship between the dose of infection and the established adult parasites (see chapter 4).

The predictions of the immigration-death model are compared to the observed data from the experiments in low responder mice and also in outbred MF1 mice (Figs. 5.8a, 5.9a). By definition, the 'high' responder mice are excluded from the analysis employing the immigration-death model owing to the existence of a significant immune response in these mice (Anderson,1976). The model provides a reasonable fit to the data from low responders exposed to low doses only during the early (0-6 weeks) part of the exposure period. Thereafter (6-12 weeks), the discrepancy between the predictions of the model and observed data becomes apparent (Fig. 5.8a). The poor fit of the model to the high dose-infection data from CBA/Ca mice and outbred MF1 mice (Fig. 5.9a) is likely to be due to reduced parasite establishment and increased parasite mortality as experience of past infection rises during the trickle infections.

5.4 Parasite establishment as a function of past experience of infection

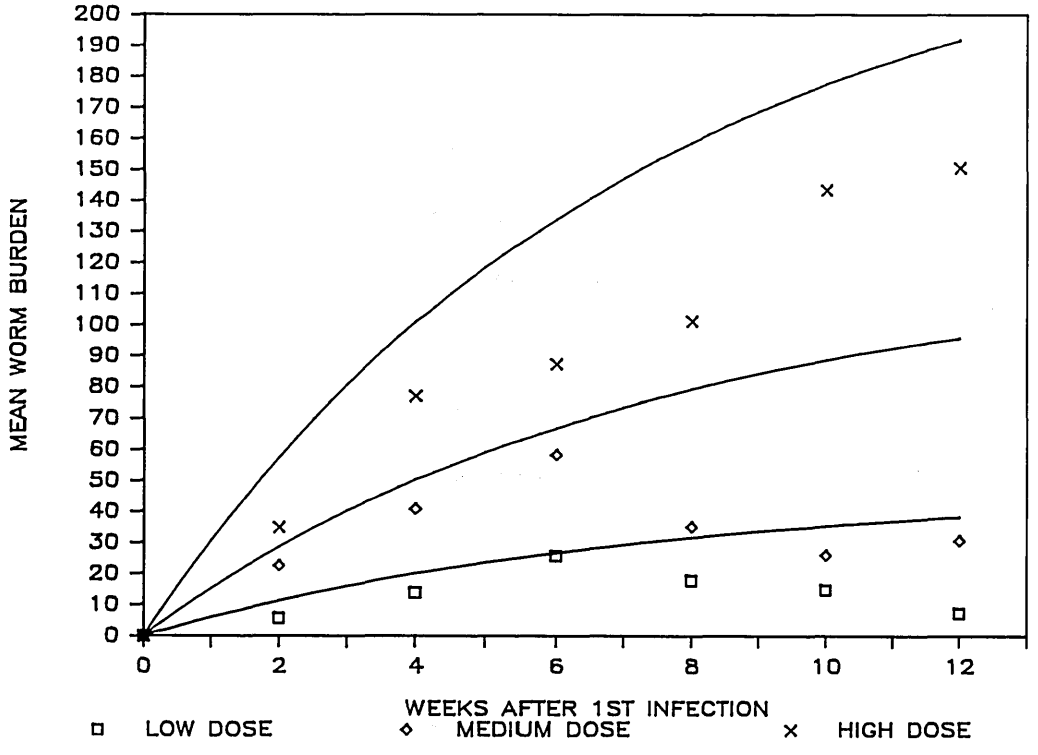
In order to investigate further the reasons for the apparent poor fit of the immigration-death model to the observed data, experiments were conducted with the aim of investigating the factors influencing the rate of establishment of parasites in hosts with differing past experiences of infection.

Figure 5.8. The observed and predicted mean worm burdens in low responder CBA/Ca inbred mice exposed repeatedly to H.polygyrus.

Groups of 10 mice were exposed to 10 (squares), 25 (diamonds) and 50 (crosses) larvae per mouse per week.

(a) Solid line - predictions of the model defined by eqns (5.1), (5.2) and (5.4) in the text. Parameter values: b = 0.66; λ_1 = 10 L3; λ_2 = 25 L3; λ_3 = 50 L3; μ = 0.14, defined per week.

(b) Solid lines - predictions of the model defined by eqns (5.4), (5.5), (5.13) and (5.14) in the main text. Parameter values: b and λ_i as defined in (a) above; μ = 0.14; β = 0.0031; σ = 0.01, all defined per week.


MEAN WORM BURDEN
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
WEEKS AFTER 1ST INFECTION
LOW DOSE
MEDIUM DOSE
HIGH DOSE

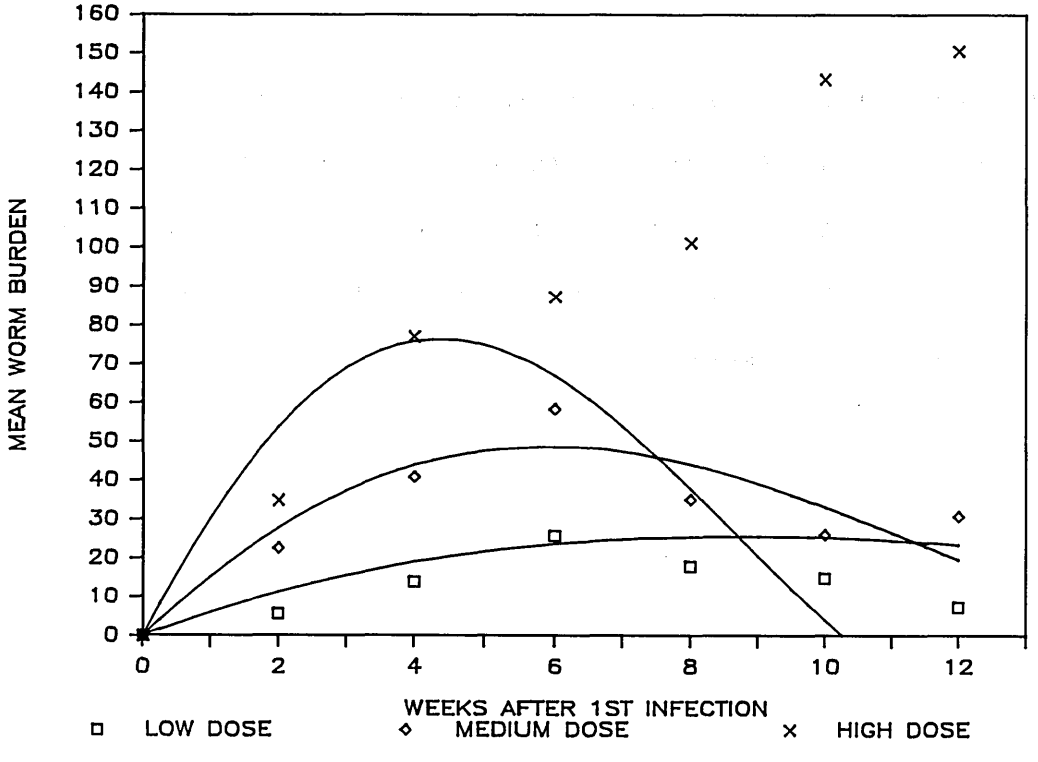
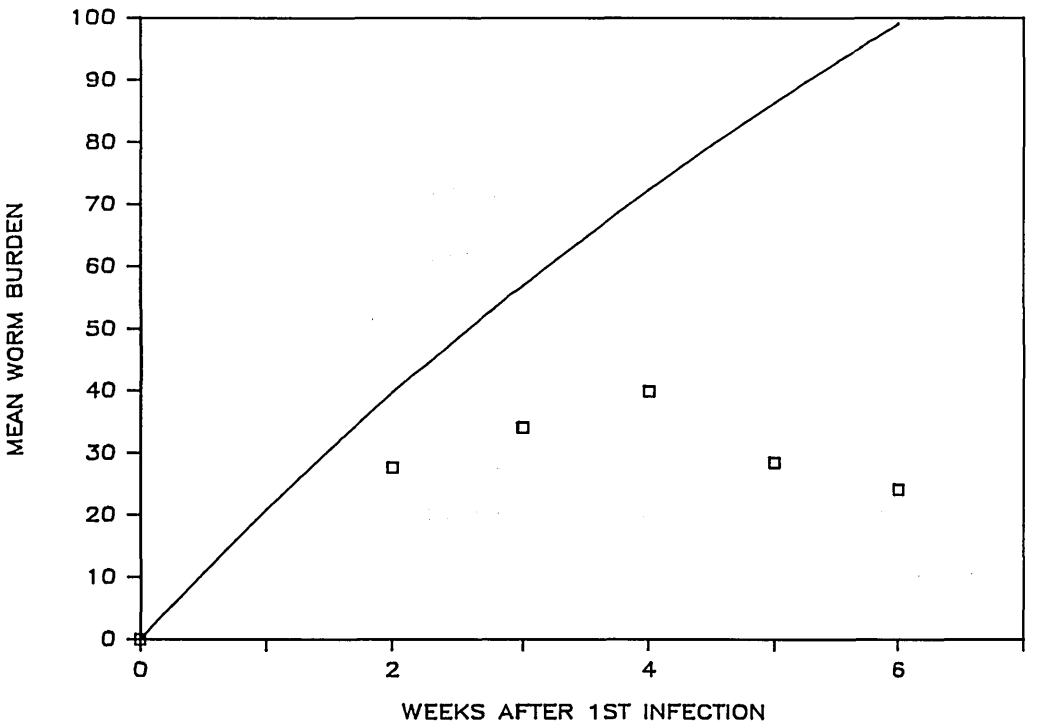

MEAN WORM BURDEN
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
WEEKS AFTER 1ST INFECTION
LOW DOSE
MEDIUM DOSE
HIGH DOSE

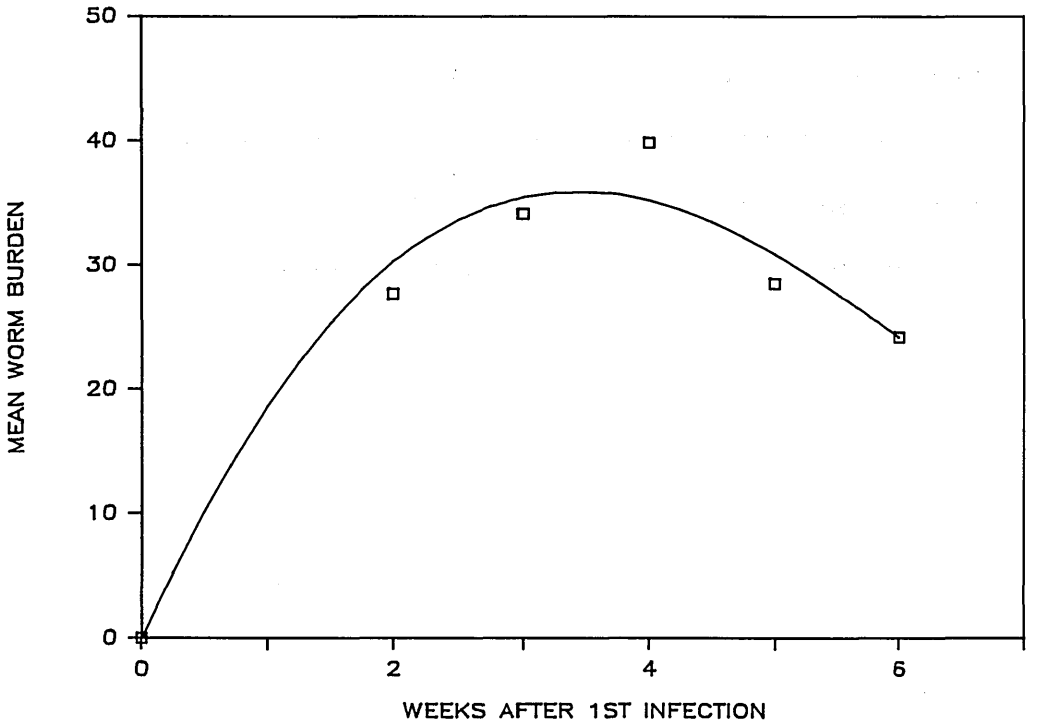
Figure 5.9. The observed and predicted mean worm burdens in outbred MF1 mice exposed repeatedly to 50 L3 H.polygyrus per mouse per week.

Groups of 8 mice were exposed to a high dose (squares).

(a) Solid line - prediction of the model defined by eqns (5.1), (5.2) and (5.4) in the main text. Parameter values: b = 0.44; λ= 50 L3; μ = 0.1005, all defined per week.

(b) Solid line - prediction of the model defined by eqns (5.5), (5.13) and (5.14) in the text. Parameter values: b and λ as for (a) above; μ = 0.29; β = 0.0063; σ= 0.01, all defined per week.


MEAN WORM BURDEN
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
WEEKS AFTER 1ST INFECTION


MEAN WORM BURDEN
50
40
30
20
10
0
0
2
4
6
WEEKS AFTER 1ST INFECTION

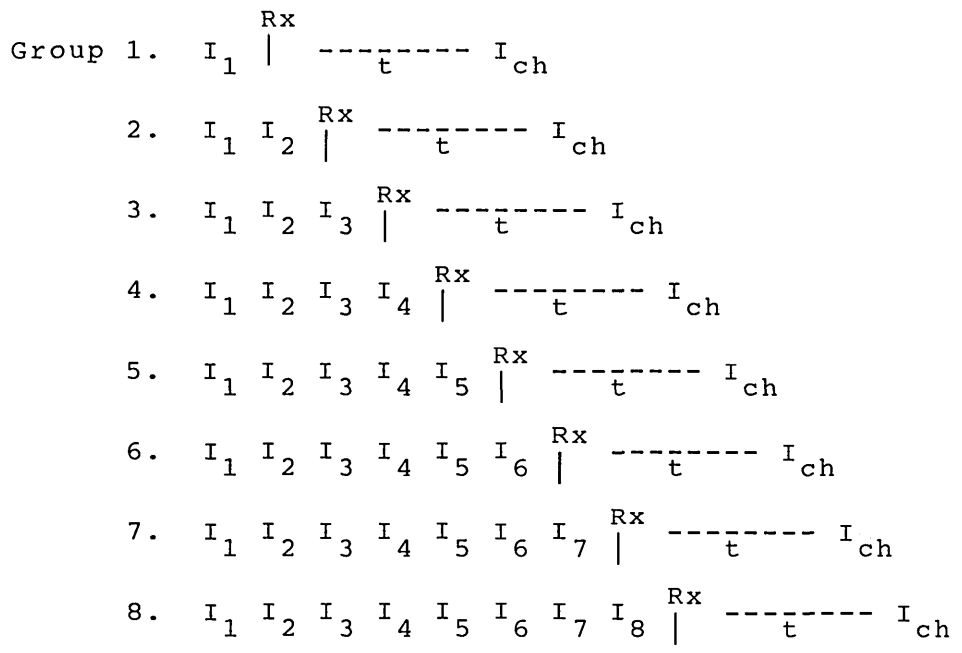
Under the conditions of repeated infection, a major problem arises in attempts to identify immigrant parasites from established or encysted parasites. This problem can be circumvented by the use of radio-labelling techniques. For example, the method of labelling challenge infection parasites with ^{14}C and their subsequent identification by autoradiography has been employed in H.polygyrus in outbred Swiss mice (Bartlett & Ball,1972). Recently, H.polygyrus larvae labelled with ^{75}Se-methionine have been used to analyse the kinetics of infection in high responder outbred LAF1 mice (Gibson, Jacobson & Sayles, 1983). ^{75}Se-methionine labelling has also been successfully used to assess the establishment of H.contortus in sheep repeatedly exposed to infection (Georgi & LeJambre, 1983; Barger, LeJambre, Georgi & Davies,1985; Barger,1986). Autoradiography suffers from the slight disadvantage that, depending on the labelling efficiency, the development of the exposed film can take a long time and the technique is cumbersome. Ideally, radioactivity can be measured from the gamma emissions of the labelled parasites using a gamma counter.

Chemotherapy has been employed to remove existing worms in order to facilitate the distinction between challenge and established worms (Michel,1969; 1970). Provided the challenge infection is given within a short time interval after drug treatment, and there is no residual effect of the drug, this approach can provide

estimates of parasite establishment. The synergistic interaction between chemotherapy and infections of H.polygyrus in mice (Kerboeuf & Jolivet,1980) and the possible relaxation of host immunosuppression following the removal of adult worms (Behnke & Parish,1979; Dobson et al,1985; Pritchard & Behnke,1985) need to be borne in mind.

Groups of inbred low responder (CBA/Ca), high responder (NIH) and outbred MF1 were exposed to 50L3 H.polygyrus/mouse/week from week 0 to week 8. One week later, 5-10 mice which had experienced the infection once only, were selected at random from each mouse strain. They were treated with ivermectin to remove the existing infection and then challenged 1 week later with 50L3/mouse. Mice given a challenge infection were killed 14 days later to assess their worm burdens. This procedure was repeated using mice given 50L3/mouse/week for 2 (i.e. 100L3), 3 (150L3), 4(200L3)... up to 8 weeks, before drug treatment, followed by a challenge infection (Fig. 5.10). Mice that were given only the drug (i.e. no previous exposure to infection) before challenge infection were employed in order to assess the residual effect of the treatment. Naive, age-matched mice of each strain served as challenge infection controls.

Figure 5.10. Design of the experiment to investigate the effect of the summed past experience of infection on parasite establishment.



Rx = treatment with ivermectin

I_i = infection with 50 L3/mouse/week (i = 1,2,3,....8)

t = time interval between treatment and challenge infection (= 1 week)

I_{ch} = challenge infection of 50 L3/mouse.

The results from the high and low responder mice are shown in Fig. 5.11, where the mean worm burdens in experimental mice are compared to those in challenge control mice. It can be seen that in NIH mice, the reduction in parasite establishment occurred early (after 2x50L3 infections) when only 10% of the challenge infection established and thereafter, mice became refractory to further infection (Fig. 5.11b). Although reduced parasite establishment was evident in CBA/Ca mice, the rate at which the establishment of the parasites was reduced was not as rapid as in NIH mice. About 10% of the challenge infection was recovered after 4x50L3 infections (Fig. 5.11a). In both mouse strains, the establishment of parasites was not affected by the change in the age of the mouse within the experimental period ($P>0.25$).

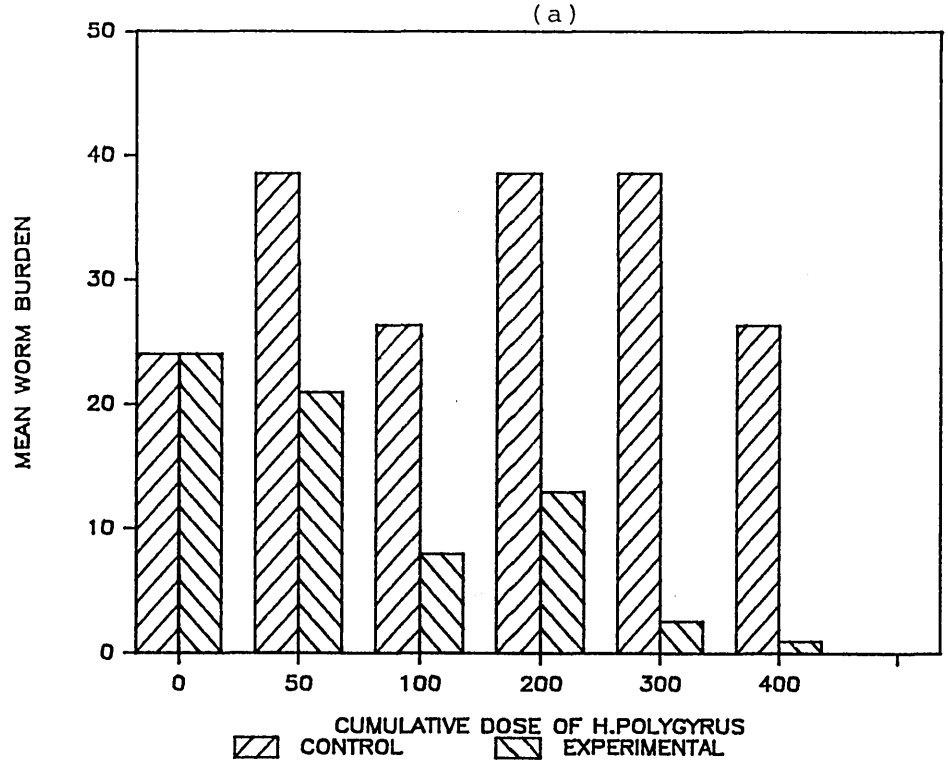
The results obtained from these experiments are compared to other studies in Fig. 5.12. It is clear that the underlying trend is for a proportional reduction in parasite establishment with increasing previous experience of infection in both inbred and outbred mice. These results suggest that the immigration-death model (Anderson,1976) should be modified to include the observed reduction in the establishment of parasites as a function of previous experience of infection. No data is available, as yet, on the effect of resistance on the death rate of the parasites during trickle infection. It is hoped that the use of radiolabelling techniques can

Figure 5.11. The mean number of challenge worms recovered from mice exposed to a repeated infection with 50L3 H.polygyrus per mouse per week (experimental) compared to the number of worms in age-matched challenge control mice, as a function of the cumulative dose of infection.

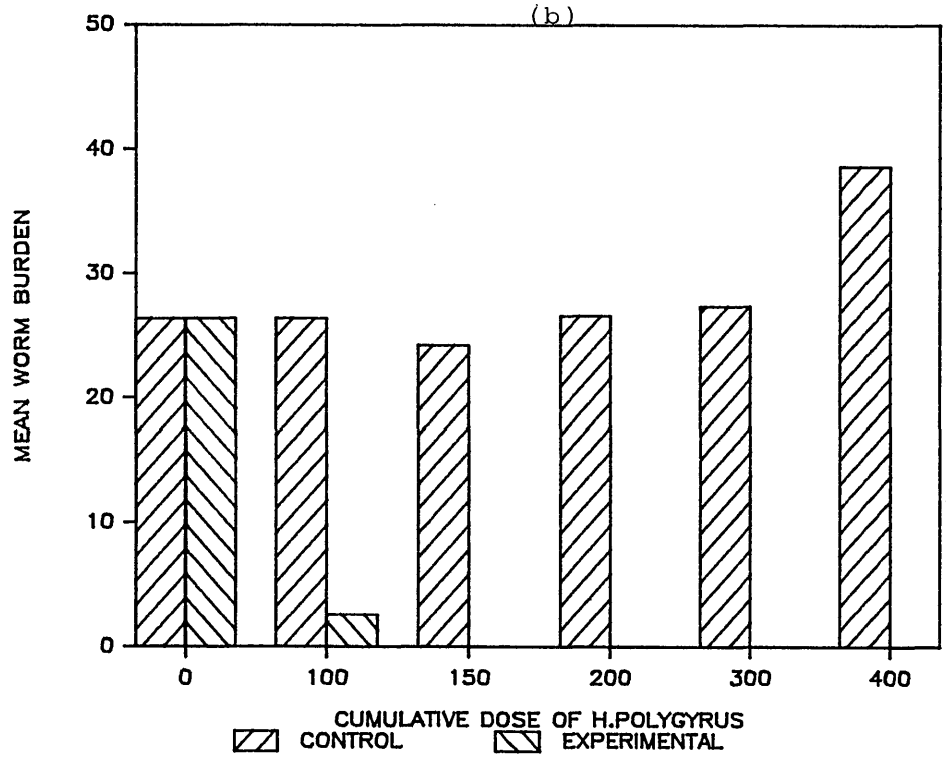
(a) Low responder CBA/Ca inbred mice;

(b) High responder NIH inbred mice.

(a)


MEAN WORM BURDEN
50
40
30
20
10
0
0
50
100
200
300
400
CUMULATIVE DOSE OF H.POLYGYRUS
CONTROL
EXPERIMENTAL

(b)


MEAN WORM BURDEN
50
40
30
20
10
0
0
100
150
200
300
400
CUMULATIVE DOSE OF H.POLYGYRUS
CONTROL
EXPERIMENTAL

provide some information on this important aspect of acquired resistance to infection. However, the short biological half-life of currently used radioactive labels poses many practical difficulties.

5.5 The acquired immunity model

The assumptions incorporated into the immigration-death model are that the immigration rate is independent of the previous experience of infection and that there are no density-dependent factors e.g. a host immune response, that influence the rate of establishment and/or death rate of worms (Anderson,1976). However, immunological responses to a variety of helminth parasites have been described in man (Ogilvie & DeSavigny,1982; Cohen & Warren,1982; Warren & Mahmoud,1984) and in other animals (Wakelin,1978; 1984; Barriga,1980).

Immune responses of vertebrates to parasitic infections act to decrease the rate of establishment and the fecundity of parasites, and to increase the rate of parasite mortality (Wakelin,1984). The precise form of the dynamic interplay between levels of herd immunity to, and rates of exposure, establishment and mortality of helminth parasites, is poorly understood (Anderson & May,1985b). Recently, mathematical models of host-helminth interactions have taken explicit account of the relationship between herd immunity and the population rate-determining parameters in the transmission of helminth

parasites (Anderson & May,1985b). The models have been applied to Ascaris lumbricoides infections in man (Anderson,1985) and Schistosoma mansoni in laboratory mice (Crombie & Anderson,1985; Anderson & Crombie,1985).

The basic model describes the rate of change in mean worm burden/host, M(t), with time t as follows:

$$dM(t)/dt = \Lambda[1 - \beta I(t)] - \mu M(t) \qquad [5.5]$$

where Λ is defined as the immigration/infection rate of parasites as in eqn [5.4], and μ is the rate of parasite mortality (per parasite per unit time); it is assumed to be constant throughout the experiment. The basic assumption of the model is that parasite establishment in the host declines linearly with the accumulated past experience of infection due to the effects of acquired resistance (I(t)) defined as

$$I(t) = \int_0^t M(t')\exp[-\sigma(t-t')]dt' \qquad [5.6]$$

where $0 \leqslant \beta I(t) \leqslant 1$.

Here β is a constant denoting the efficacy of the acquired resistance as it acts to reduce parasite establishment. The constant, σ, is defined as the persistence of the acquired resistance ('immunological memory') in the host following the induction of the resistance by the parasite. The term $1/\sigma$ defines the average duration of the host resistance i.e. when $\sigma \rightarrow 0$, the resistance is long-lived

and when $\sigma \rightarrow \infty$, it is short-lived. Experimental assessment of the duration of resistance in Swiss-Webster outbred mice (high responders) indicated a duration of 50 weeks, with 99% protection recorded in female mice (Van Zandt, Cypess & Zidian,1973). Thus, the value of σ is probably less than 0.02. Using a trickle infection design, the degree of acquired resistance (as measured by parasite establishment) was found to be 95% at 7 weeks in low responder CBA/Ca mice (see section 5.9).

The model defined in equation[5.5] can be modified to take into account the observed heterogeneity in immunological responsiveness to helminth parasites in laboratory animals (Wakelin,1985a,b). Genetic variability in immunity to parasites has been described in outbred mice (Wakelin,1985b). A model described by Anderson & May (1985b) and Anderson (1985) includes the interaction between heterogeneity in 'immunological responsiveness' and exposure to infection by considering a population consisting of a proportion f_1 of 'high' and f_2 of 'low' responder phenotypes. The total population is given by

$$f_1 + f_2 = 1 \qquad [5.7]$$

$M_1(t)$ and $M_2(t)$ are defined as the mean worm burdens of the high and low responder individuals, respectively.

The equilibrium mean worm burdens in these two types of individuals can be described by a generalisation of equation [5.5] as follows:

$$dM_1(t)/dt = \Lambda[1-\beta_1 I_1(t)] - \mu_1 M_1(t) \qquad [5.8]$$

$$dM_2(t)/dt = \Lambda[1-\beta_2 I_2(t)] - \mu_2 M_2(t) \qquad [5.9]$$

where the subscripts 1 and 2 refer to the high and low responders and

$$I_1(t) = \int_0^t M_1(t)\exp[-\sigma_1(t-t')]dt' \qquad [5.10]$$

$$I_2(t) = \int_0^t M_2(t)\exp[-\sigma_2(t-t')]dt' \qquad [5.11]$$

Note that the average mean worm burden at time t in the outbred (mixed) population will be given by

$$M(t) = \sum_{i=1}^{2} f_i M_i(t) \qquad [5.12]$$

The solution of the acquired immunity model (eqn 5.5) is given by Anderson & May,1985(b) as follows. For $4\beta\Lambda < (\mu-\sigma)^2$,

$$M(t) = A + \Lambda/\lambda[(p_1+\sigma)/p_1\exp(p_1 t) - (p_2+\sigma)/p_2\exp(p_2 t)] \qquad [5.13]$$

where $A = \Lambda\sigma/(\sigma\mu + \beta\Lambda)$; $p_1 = [-(\mu+\sigma-\lambda)]/2$;

$p_2 = -(\mu + \sigma+\lambda)/2$ and $\lambda = [(\mu - \sigma)^2 - 4\beta\Lambda]^{\frac{1}{2}}$.

When $4\beta\Lambda > (\mu - \sigma)^2$, then the solution of eqn [5.5] is

$$M(t) = A + \exp(-\mu+\sigma)^{t/2}[2A/\theta \sin(\frac{t\theta}{2}) [1-A(\mu+\sigma)/2\Lambda] - A \cos (\frac{t\theta}{2})] \qquad [5.14]$$

Depending on the choice of parameter values, the model is able to generate many patterns of change in mean worm burden with time (or host age). Model predictions are compared with the observed data in Figs. 5.8b and 5.9b. The closeness of fit of the model to the observed data in outbred mice (Fig. 5.9b) suggests that MF1 outbred mice consist of a larger proportion of 'high' responders than 'low' responders. The model also provides a satisfactory fit to the data from low responder CBA/Ca mice exposed to the lower doses of infection but fails to mimic the dynamics of worm burden change in CBA/Ca mice given the higher dose of 50L3/mouse/week (Fig. 5.8b). Given certain parameter values, the model predicts a monotonic rise in mean worm burdens to a plateau at low intensity of infection and a convex pattern at high rates of exposure with mean worm burdens declining with increasing time of infection (Anderson & May,1985b). The discrepancy between the convexity predicted by the model at high doses of infection in low responder mice and observed data is probably a result of high dose immunosuppression.

The model also fails to mirror the data from high responder mice (although it is able to mimic the overall pattern of infection) when a crude estimate of the constant parasite death rate (μ=0.1) obtained from single infections is employed. Future generations of mathematical models must include greater non-linearity in the reduction in parasite establishment as a function of past experience of infection (Fig. 5.12).

5.6 A new model incorporating reduced parasite establishment, increased parasite death rate and host immunosuppression

A mathematical model based on the salient features of the population biology of *H.polygyrus* in laboratory rodents is needed. First, the proportional reduction in parasite establishment appears to be a linear function of the summed experience of past infection in mice (Fig. 5.12). Secondly, the crude estimate of parasite mortality rate obtained from single infection experiments underestimates the additional death rate due to the effects of resistance acquired during repeated exposure (see, for example, Fig. 5.1b). Although parasite survivorship in challenge infections is similar to that observed in naive mice (chapter 4), the repeated infection regime is likely to cause an increased immune response since fourth-stage larvae are immunogenic in mice (Behnke & Parish,1981; Prowse *et al*,1979; Jacobson *et al*,1982). The effect of an increased immune response on the observed rapid expulsion(RE) and exponential loss of gastrointestinal worms because of a host mucosal, immuno-inflammatory response has been

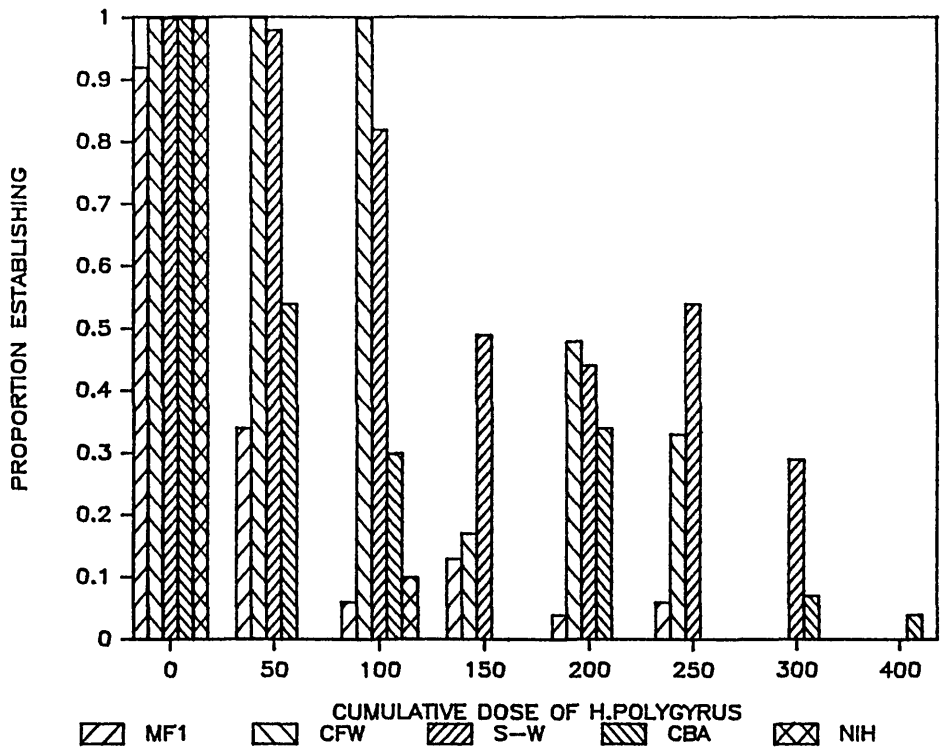


Figure 5.12. The observed mean number of H. polygyrus establishing from a challenge infection of 50 L3/mouse, as a proportion of the mean number of parasites recovered from challenge control mice. The graph compares data from inbred and outbred mice.

i) outbred MF1 mice - this study;

ii) outbred CFW mice - Bartlett & Ball (1972);

iii) outbred S-W mice - Cypess, Van Zandt & Zidian (1973);

iv) inbred CBA/Ca mice - this study;

v) inbred NIH mice - this study.

reviewed by Miller (1984; 1986). Thus, parasite mortality rate is likely to increase during repeated exposure of mice. Third, adult H.polygyrus have been observed to immunosuppress certain strains of mice (Behnke et al,1983; Pritchard & Behnke,1985; Dobson & Cayzer,1983; Chapman et al,1979a,b). The immunosuppression is dose-dependent (Dobson et al,1985; Sitepu et al,1985) and facilitates the accumulation of immigrant parasites and/or persistence of other parasites such as Trichuris muris (Behnke, Ali & Jenkins,1984; Jenkins & Behnke,1977); Trichinella spiralis (Behnke, Wakelin & Wilson,1978; Hagan & Wakelin, 1982); Nippostrongylus brasiliensis (Jenkins,1975) and Hymenolepis diminuta (Alghali, Hagan & Robinson,1985).

A model which takes specific account of the interplay between these three effects describes the change in mean worm burdens with time, M(t), as follows:

$$dM(t)/dt = \Lambda[1-\beta_1 I(t)] - \mu M[1+\beta_2 I(t)] \qquad [5.15]$$

where the parameters Λ, β, μ, are as described before and I(t) is as defined in eqn [5.6]. The subscripts 1 and 2 refer to the strength of acquired immunity as it affects immigration and death rates of the parasite, respectively.

To include the effect of host immunosuppression on parasite establishment, the immigration term in eqn [5.15] can be formulated as follows:

$$\Lambda[1-(\beta-\alpha)I(t)] \qquad [5.16]$$

with the constraint, $0 \leqslant 1-(\beta-\alpha)I(t) \leqslant 1/b$, and b is as defined in eqn[5.4] and α is a constant defining the efficacy of the immunosuppression. The decay in immunosuppression after removal of adult worms is assumed to be linear with time and has been estimated to last for 7 weeks (Dobson et al, 1985). When α is very small compared to β (e.g. in high responder mice), the immigration term in eqn [5,16] becomes $\Lambda(1-\beta I(t))$ as in eqn [5.13]. As α (=immunosuppression) increases (e.g. low responder mice), so the model moves towards the immigration-death model (eqn 5.5), and predicts an increase in mean worm burden with time to a stable plateau. When $\alpha > \beta$, the worm burden will rise for longer, and eventually reach a plateau above that for constant immigration-death. The value of β can be crudely estimated (assuming $\sigma \doteqdot 0$) from a plot of the summed experience of infection, $\int_0^t M(t')dt'$, against the proportion of challenge parasites establishing, $(1-\beta I(t))$, where the slope of the linear relationship approximates β. The cumulative dose was used as an approximation of $\int_0^t M(t')dt'$. For the high responder NIH, the value of β was estimated as 0.01 and for the low responder CBA/Ca, $\beta \doteqdot 0.003$. The experimental design does not provide an estimate of the increased death rate (μ') due to acquired resistance. A best fit (by non-linear statistical procedures (Conway, Wilcox & Glass,1970)) μ' estimated from all the data on NIH mice was found to be $\mu' = 0.7$.

The predictions of this model provided a satisfactory fit to the data from high responder mice (Fig. 5.13), where the mean worm burden was obtained by numerical solution of eqn [5. 5]. With only Λ adjustable for the three levels of infection, the model is able to crudely portray the essential features of the patterns of change in worm burdens. At low doses of infection, the mean worm burden rose monotonically to a plateau. A convex age-intensity curve was obtained at the high dose of infection. The model also captures the interesting observation that as the period of exposure increased, the mean worm burdens in mice exposed to the high dose were lower than those obtained from mice given low doses of infection despite repeated infection of mice. Thus, low mean intensity of parasites in 'older' age groups of the population need not reflect reduced exposure to infection. Indeed, it may reflect the converse situation (Fig. 5.13).

5.7 The dynamics of trickle infections after chemotherapy

Drugs of high efficacy against helminth parasites are available (Van den Bossche,1978; Rogers,1986). Aside from biochemical and resistance problems associated with the development and use of drugs (Wang,1982), knowledge of their effective use to control helminth infections in human and animal communities is limited at present (Anderson & May,1982; Anderson & Medley,1985). The problem is exercebated by the observed rapid re-infection rates in

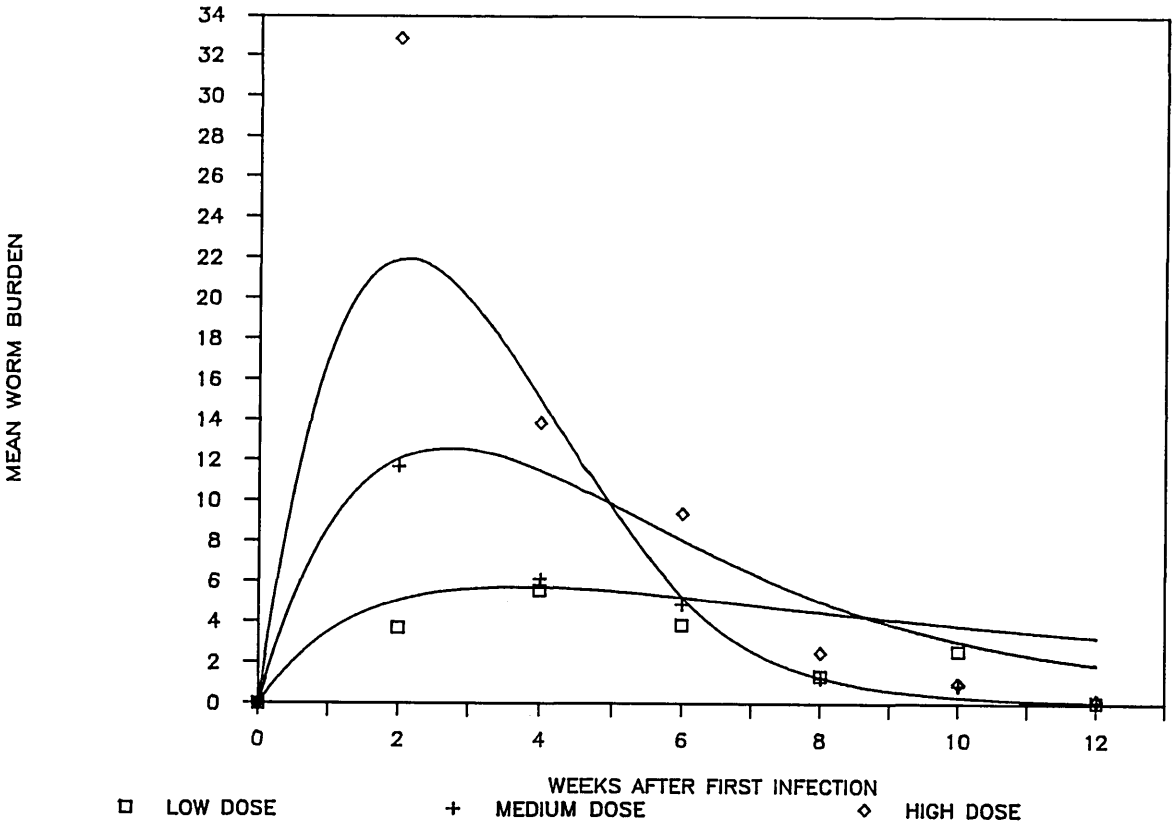


Figure 5.13. The observed and predicted mean worm burdens in inbred high responder NIH mice exposed repeatedly to H. polygyrus.

Groups of 10 mice were exposed to 10 L3 (squares), 25 L3 (crosses) and 50 L3 (diamonds) per mouse per week.

Solid lines - predictions of the model defined by eqns [5.4] and [5.5] in the main text. Parameter values: b = 0.57; λ_1 = 10 L3; λ_2 = 25 L3; λ_3 = 50 L3; μ = 0.71; β = 0.0117; σ = 0.01, all defined per week.

areas endemic for intestinal nematode infections (Anderson & May,1985a). It is apparent, therefore, that better strategies are needed to combat helminth infections by chemotherapy (Warren,1981). Mathematical models which conceptualize the dynamic interaction between hosts and parasites can be helpful in the design of such control programmes (Anderson & May,1982; 1985 a,b; Anderson,1985; Anderson & Medley,1985).

In this section, the significance of chemotherapy in mice exposed to trickle infections with H.polygyrus is explored. Drug treatment of mice infected with H.polygyrus has several consequences. First, and obviously, chemotherapy increases the mortality rate of parasites. Secondly, studies based on primary and challenge infections in mice have demonstrated the development of acquired resistance to infection following chemotherapy (Behnke & Wakelin,1977; Cypess & Zidian,1975; Kerboeuf & Jolivet,1980), which may be linked to the genetic background of the mice (Wakelin,1985b). Third, removal of adult worms by chemotherapy has the effect of reducing the host immuno-suppression (Pritchard & Behnke,1985; Dobson et al,1985).

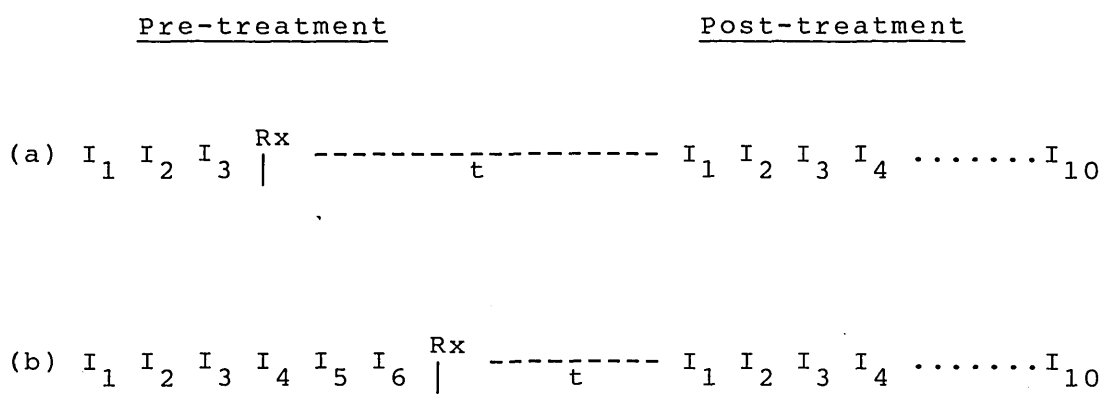
Apart from the work of Kerboeuf & Jolivet (1980) using outbred CD1 mice exposed to increasing infections of H.polygyrus, studies on the interaction between chemotherapy and repeated infections have not been described, despite the importance of such research. By treating a host population, the level of resistance may be reduced

if the maintainance of immunity is dependent on the presence of worms in the host.

Experiments using high responder NIH and low responder CBA/Ca mice were performed in order to assess the impact of chemotherapy on the dynamics of repeated infections. Two hundred and forty (240) CBA/Ca female mice were divided into two groups of 120 mice each. Group I mice received initial trickle infections for a period of 3 weeks at the low rate of 10L3/mouse/week (60 mice) and a high rate of 50L3/mouse/week (60 mice) before drug treatment. One week after treatment, all mice were exposed to a second round of trickle infection at the same rate as before chemotherapy, for a further 10 weeks. At 2-weekly intervals, 10 mice from each dose group were selected at random and killed to determine their worm burdens after faecal parasite egg counts from individual mice. Group II mice (120 mice) were treated similarly to Group I mice, except that the initial trickle infection was extended to a period of 6 weeks (Fig. 5.14). The faecal parasite egg counts in one group of 10 mice in Group II mice exposed to the high dose of 50L3/mouse/week were monitored at approximately 2-weekly intervals.

Similar experiments showed that NIH mice given a trickle infection and then treated, were all completely refractory to further infection. The results in Fig. 5.15 suggest that low responder CBA/Ca mice were partially resistant to further infection following <u>early</u> (3 wk) drug

Figure 5.14. Experimental design to investigate the impact of chemotherapy on the dynamics of repeated infection of low responder mice with H. polygyrus at the low (10L3/mouse/wk) and high (50L3/mouse/wk) doses of infection.



I_i = Infection with 10 or 50 L3/mouse/week (i=1,2,3,...10)

Rx = treatment of mice with ivermectin

t = time interval between treatment and re-trickle infection (= 1 week).

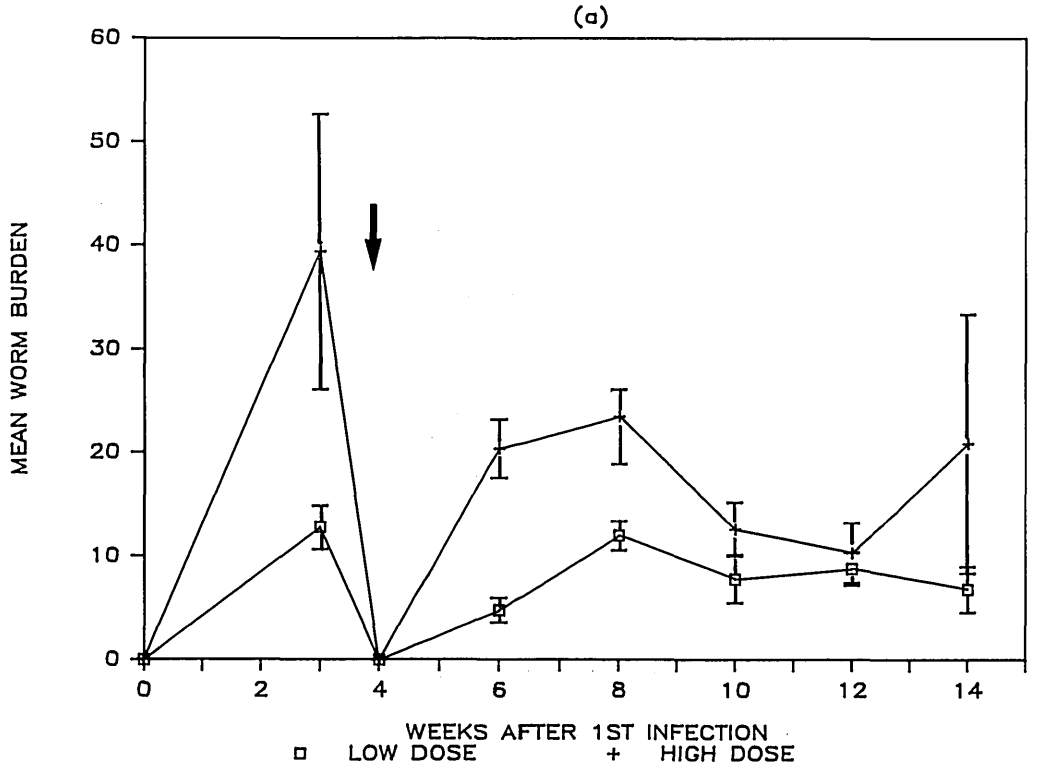
treatment. The mean worm burdens after drug treatment were significantly lower than the pre-treatment mean worm burdens only in mice exposed to the high dose of infection ($P<0.01$). There was no significant difference between the pre- and post-treatment mean worm burdens, at all times during the infection, in mice exposed to the low dose of infection ($P>0.05$)(Fig. 5.15a). The post-treatment mean worm burdens in mice given the high dose of infection were sometimes significantly higher than those given the low infection dose ($P(6wk)=0.0002$; $P(8)=0.038$; $P(10)=0.186$; $P(12)=0.66$; $p(14)=0.29$). Mice which had a longer (6wk) exposure to trickle infection prior to drug treatment accumulated worms in a dose-dependent manner (Fig. 5.15b). Following treatment, however, all mice were highly resistant despite continued exposure as judged by the significantly lower post-treatment mean worm burdens compared to pre-treatment worm burdens ($P<0.001$). The differences between the high and low dose groups in post-treatment worm burdens was not consistently significant ($P(8wk)=0.0002$; $P(10)=0.076$; $P(12)=0.237$; $P(14)=0.033$; $P(16)=0.028$).

The per capita fecundity of worms recovered from these experiments are shown in Fig. 5.16. The reproductive rate of H.polygyrus was similar between pre- and post-treatment counts in mice exposed to an early (3 wk) initial trickle infection (Fig. 5.16a). The fecundity was markedly convex in shape. However, this pattern does not closely mimic the observed age-dependent parasite fecundity (see chapter 4).

Figure 5.15. The observed mean worm burdens (±S.E.) in groups of 10 low responder CBA/Ca mice exposed to a 3-week (a) and a 6-week (b) initial trickle infection before the application of chemotherapy (arrow).

Squares - low dose (10L3 *H.polygyrus*/mouse/week);

Crosses - high dose (50L3 *H.polygyrus*/mouse/week).


(a)
MEAN WORM BURDEN
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
WEEKS AFTER 1ST INFECTION
LOW DOSE
HIGH DOSE

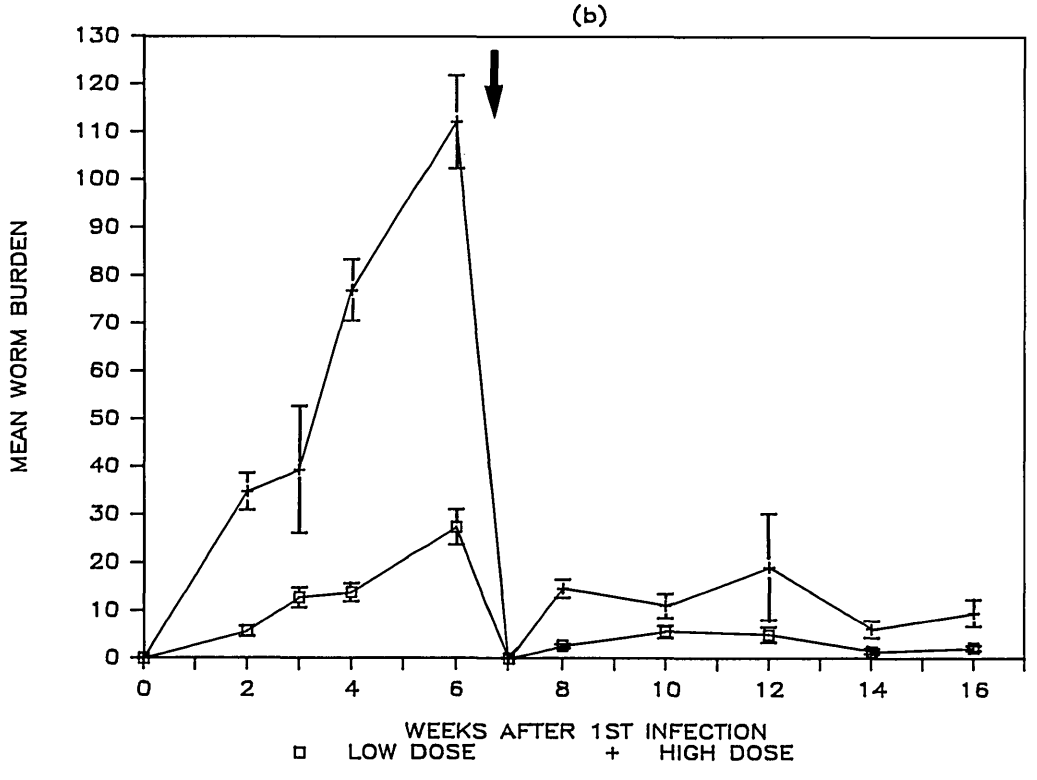
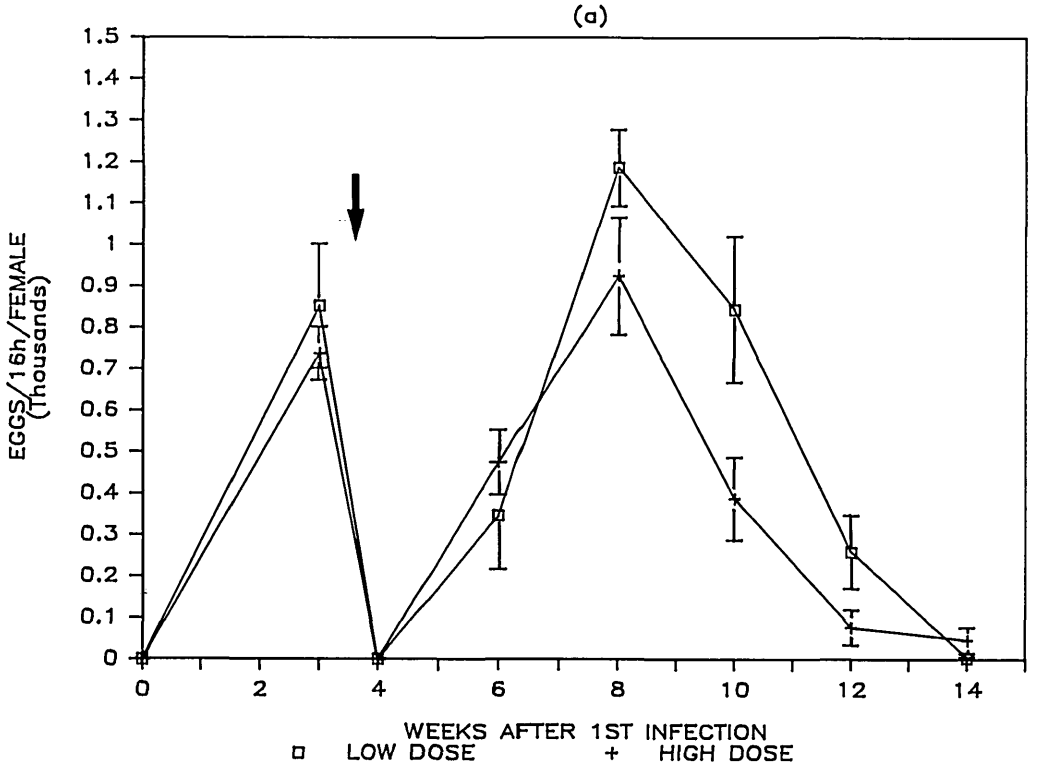
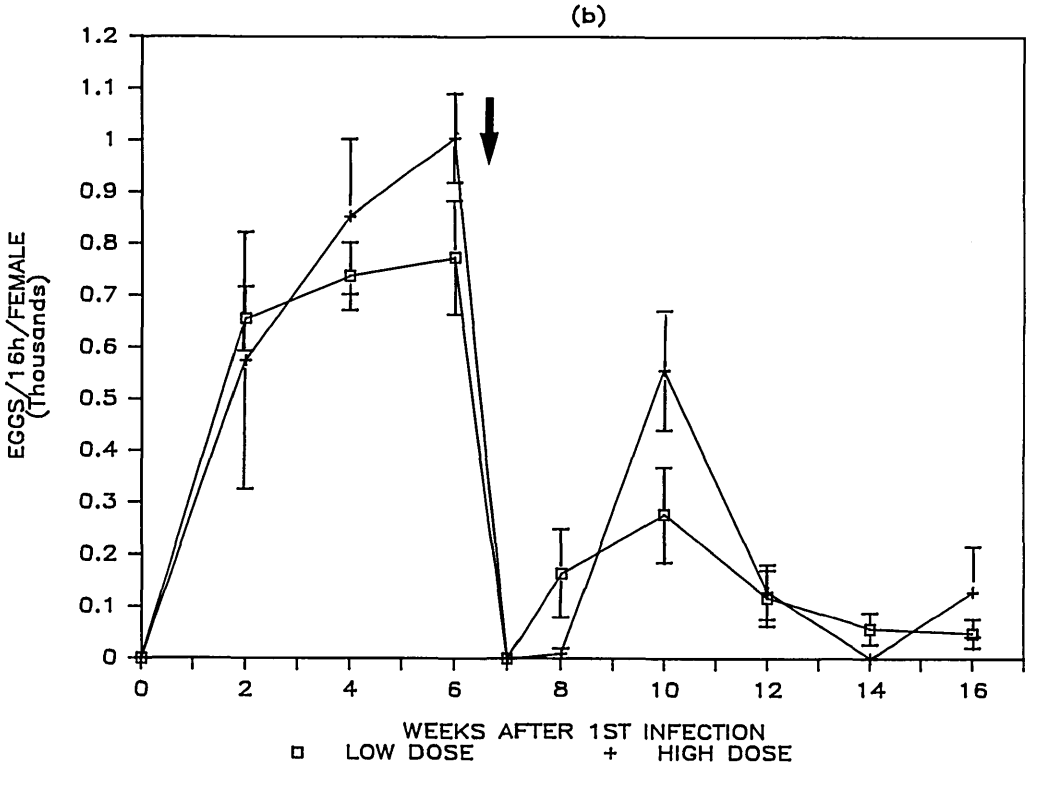

(b)
MEAN WORM BURDEN
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
WEEKS AFTER 1ST INFECTION
LOW DOSE
HIGH DOSE

Figure 5.16. The per capita fecundity (±S.E.) of H.polygyrus from groups of 10 low responder CBA/Ca mice exposed to a 3-week (a) and a 6-week (b) initial trickle infection before chemotherapy (arrow).

Squares - low dose of 10 L3/mouse /week;

Crosses - high dose of 50 L3/mouse/week.


(a)
EGGS/16h/FEMALE (Thousands)
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
WEEKS AFTER 1ST INFECTION
LOW DOSE
HIGH DOSE


(b)
EGGS/16h/FEMALE (Thousands)
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
16
WEEKS AFTER 1ST INFECTION
LOW DOSE
HIGH DOSE

Following a longer trickle infection before treatment, the fecundity of H.polygyrus was reduced considerably throughout the experimental period (Fig. 5.16b). This can be interpreted as the effect of acquired resistance induced gradually by a longer experience of infection on the fecundity of the worm. This hypothesis is clearly supported by the evidence in Fig. 5.17 which shows the results of total eggs per mouse recovered from the same individuals over the 16-week period of trickle infection plus chemotherapy.

5.8 The impact of vaccination on repeated infections

The potential for vaccination against helminth parasites of man and animals has been discussed by a number of people in recent years (Lloyd,1981; Miller,1978; Clegg & Smith,1978; Newell,1985; Smithers,1986;Simpson,1986). The formulation of ideal strategies for the use of vaccines to control helminth infections on a community level has also benefitted from recent mathematical studies (Anderson & May,1982; 1985c; Anderson,1985). To be successful, a vaccine must be able to, inter alia, confer long-lasting immunity to susceptibles despite continued exposure to infection and to achieve a high level of conversion of non-responders to responders. Essentially, vaccines to gastrointestinal parasites must be able to induce local immuno-inflammatory protective responses (Miller,1986).

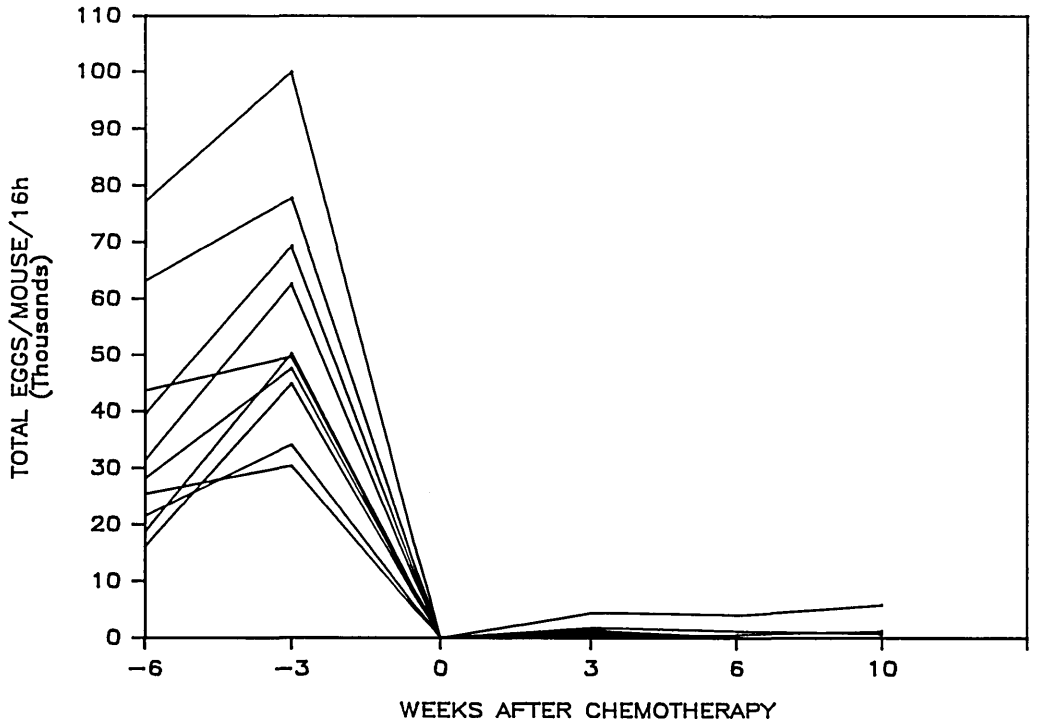


Figure 5.17. Changes in the total number of H. polygyrus eggs excreted by same mice exposed to 50 L3/mouse/week for 6 weeks before chemotherapy and 1 week thereafter, following infection for a further 10 weeks. Ten (10) mice were monitored.

Irradiated larvae have been employed as vaccines against the lungworm of cattle, Dictyocaulus viviparous (Clegg & Smith,1978) and the dog hookworm, Ancylostoma caninum (Miller,1978). Irradiated H.polygyrus larvae induce immunity in mice (Hagan, Behnke & Parish,1981; Behnke & Robinson,1985) although the precise mechanism of induction of resistance is not well understood (Behnke, Parish & Hagan,1980).

The efficacy of oral vaccination of high (NIH) and low (CBA/Ca) responder mice with irradiated H.polygyrus larvae (courtesy of Dr. J. Behnke, Nottingham University) and the resultant protection to repeated infection with normal infective larvae in these two mouse strains was assessed. Ten (10) CBA/Ca female mice and 11 female NIH mice were each immunized by infection with 250 irradiated larvae on week 0. One week later, all 21 mice were exposed to 50L3 H.polygyrus/mouse/week for 6 weeks. Faecal parasite egg counts were performed from individual mice in order to monitor the infection. Infectivity of irradiated and normal larvae was checked by infection of 10 mice with 250 irradiated larvae and 10 mice with 250 normal larvae. Five mice from each infectivity control group were killed at 2 weeks post-infection and the other five mice were killed at the same time as the mice in the experimental groups (i.e. 7 weeks) to check the survivorship of irradiated worms. Ten CBA/Ca and 10 NIH mice were also given 50 normal L3/mouse /week at the same time as the vaccinated mice to serve as 'trickle' controls.

The results presented in Fig. 5.18 show that vaccination of high responder conferred even greater resistance to infection ($P=0.015$) although mice that were not vaccinated were also >95% protected. This result appears to support the suggestion made earlier, that the genetic background and the phenotype of a mouse strain chosen for dissection of mechanisms of resistance should be taken into account. Interestingly, CBA/Ca (low responders) mice were also significantly protected compared to non-vaccinated mice ($P=0.0034$). The level of protection attained by CBA/Ca mice was, however, lower than that observed in high responder NIH mice ($P<0.0001$).

Examination of parasite egg counts from individual mice (especially in CBA/Ca) with high initial parasite burdens (indicated by high egg counts) suggested that these mice always had high parasite burdens throughout the experimental period (as tested by the Spearman's rank correlation method) probably due to variability induced by the exposure technique (Fig. 5.19). This observation may hint at the operation of chance events (e.g. the probability of a host being exposed initially to high infection which induces immunosuppression) in determining the level of response to subsequent infections. Further work is needed to investigate this observation.

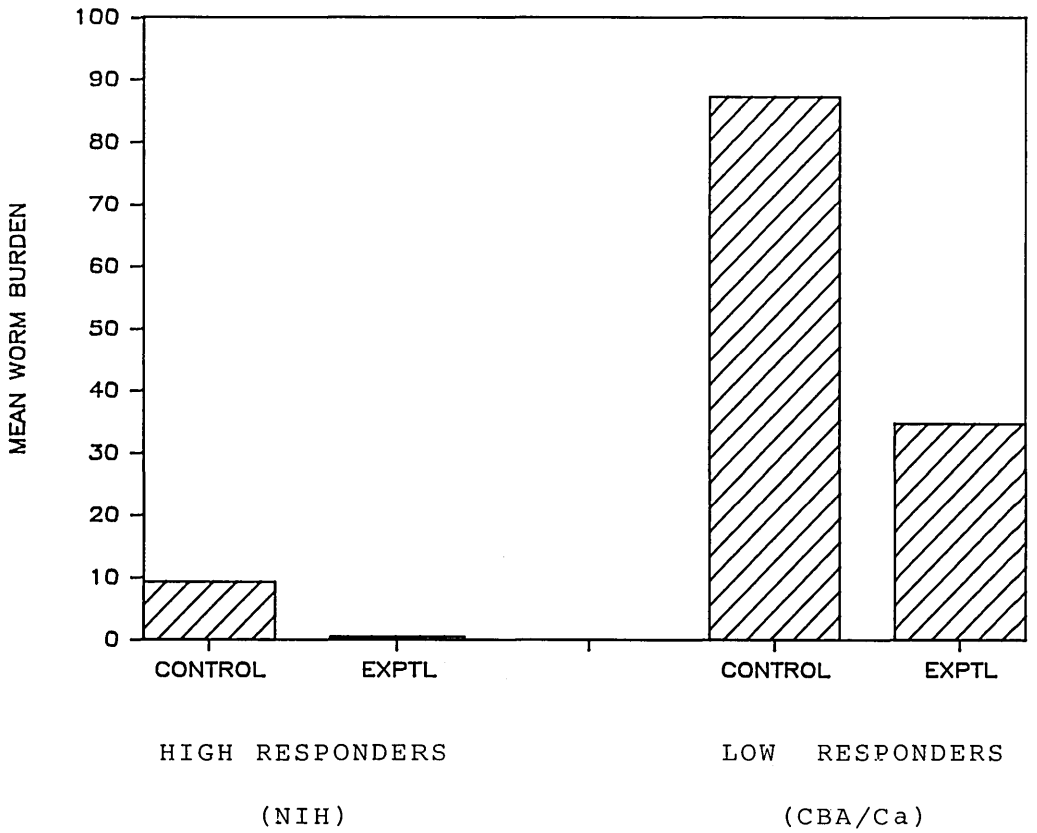


Figure 5.18. The impact of vaccination of low and high responder inbred mice with 250 irradiated H. polygyrus larvae on the dynamics of repeated infection with normal larvae at the rate of 50 L3/mouse/week.(n = 10-11 mice).

Mean worm burdens (±SEM) in control mice given a single dose of 250 irradiated larvae at 2 weeks p.i. = 29.8 ± 5.7; at 7 weeks p.i. = 2.2 ± 0.6.

Figure 5.19. Changes in the total egg production of H.polygyrus/16h/mouse in individual high (a) and low (b) responder mice repeatedly infected with 50 L3/mouse/week, one week after vaccination with 250 irradiated larvae. (Irradiated worms did not produce any eggs).

Spearman rank correlation co-efficients:

(a) NIH: wk 2 vs wk 3 - r_s = 0.07; P>0.50

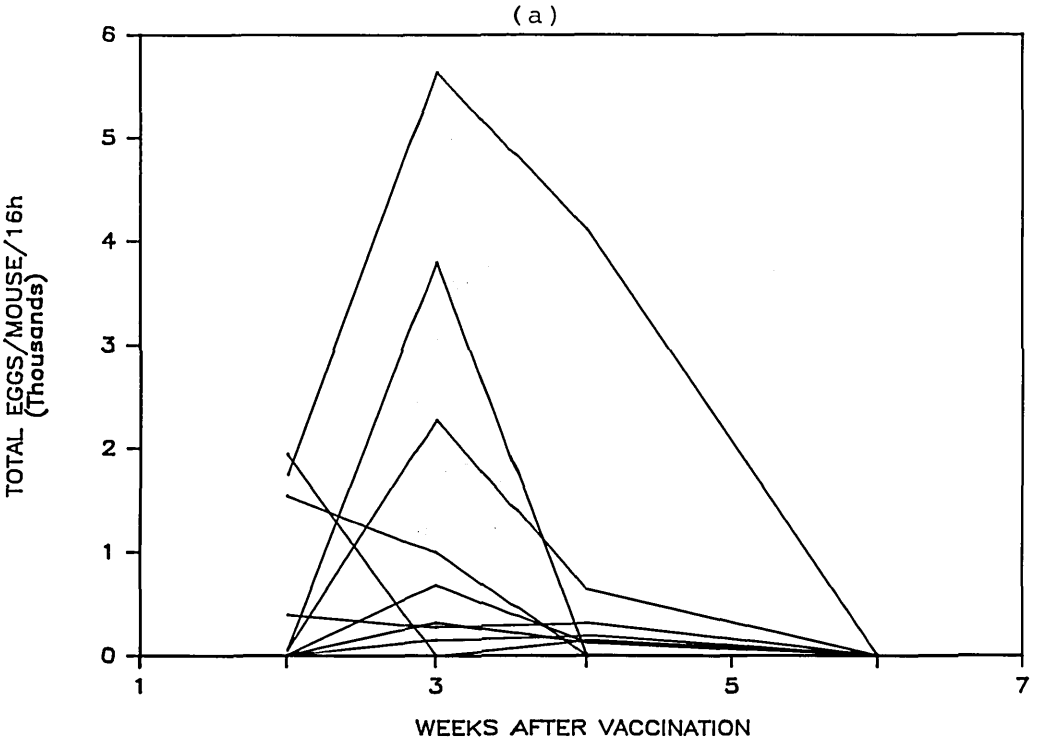
wk 2 vs wk 4 - r_s =-0.22; P>0.50

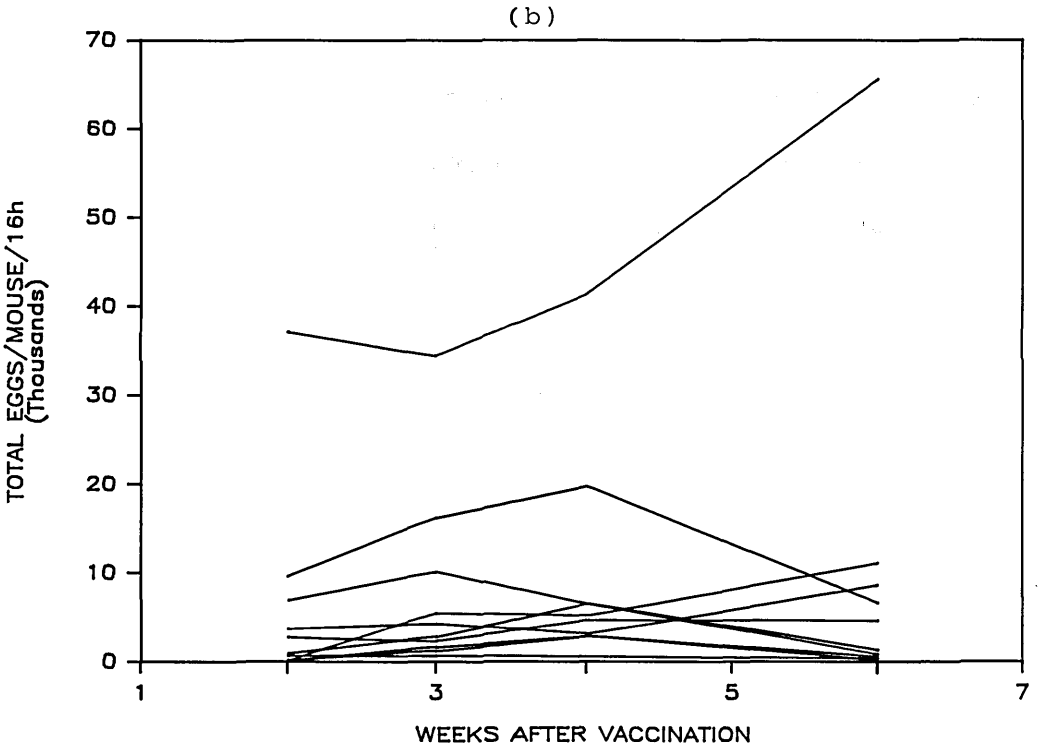
wk 2 vs wk 6 - r_s = 0.34; P>0.50

(b) CBA: wk 2 vs wk 3 - r_s = 0.71; P<0.05

wk 2 vs wk 4 - r_s = 0.67; P<0.05

wk 2 vs wk 6 - r_s = 0.51; P>0.20


(a)
TOTAL EGGS/MOUSE/16h (Thousands)
0
1
2
3
4
5
6
1
3
5
7
WEEKS AFTER VACCINATION


(b)
TOTAL EGGS/MOUSE/16h (Thousands)
0
10
20
30
40
50
60
70
1
3
5
7
WEEKS AFTER VACCINATION

5.9 The duration of resistance in mice repeatedly exposed to infection

The significance of chemotherapy (section 5.7) and vaccination (section 5.8) as determinants of the ability of mice to acquire resistance was investigated by repeatedly exposing mice to an initial trickle infection followed by a second round of trickle infection after a short interval of one week following chemotherapy or vaccination. Drug control tests showed that ivermectin did not have a residual effect on subsequent H.polygyrus infections given as soon as 7 days after treatment (see Fig. 5.11). In order to assess the duration of resistance, it is necessary to extend the interval between chemotherapy and the second trickle exposure.

Few studies have attempted to assess the duration of acquired resistance, despite its obvious importance. Moreover, the immunological basis for this resistance remains poorly understood at present (Palmas, Bortoletti, Conchedda & Gabriele,1986; Wakelin, Grencis & Donachie, 1982), although T-memory cells are thought to be involved (Palmas et al,1986). Studies using single and challenge infections on the long term duration of the rapid rejection of Trichinella spiralis by rats have indicated that this capacity persists for up to 18 months (Bullick, Russell & Castro,1984). In mice, the ability to reject T.spiralis infective larvae wanes within a few

weeks (Wakelin & Lloyd,1976). Hymenolepis nana primary infections in mice also confer protection against homologous challenge infection for up to 180 days (Palmas et al,1986). The study of the duration of resistance to H.polygyrus in Swiss outbred mice immunized by 3 weekly infections of 50L3/mouse/week, followed by chemotherapy, have shown that protection levels to a single challenge of up to 99% persisted for at least 50 weeks in female mice (Van Zandt et al,1973).

Forty (40) female CBA/Ca mice were divided into 4 groups of 10 mice each. Group A mice were further divided into 5 age-matched control and 5 experimental mice. The experimental mice were exposed to a repeated infection of 50L3 H.polygyrus/mouse/week for 3 weeks, then treated with ivermectin to remove all stages of the parasite. The second round of trickle infection of the same number of both experimental and naive mice was continued again after 1 week following drug treatment, for a further 4 weeks. Groups B, C and D were re-trickled after 3, 5 and 7 weeks interval between chemotherapy and second round of trickle infection, respectively (Fig. 5.20). A further group of 5 mice vaccinated with 250 irradiated larvae was exposed to trickle infection 7 weeks after vaccination.

Figure 5.20. Experimental design to assess the duration of resistance induced by repeated infection plus chemotherapy and vaccination of low responder mice during repeated exposure of mice to 50 L3 H.polygyrus/mouse/week.

	Pre-treatment		Post-treatment/vaccination
A.	$I_1\ I_2\ I_3$	Rx | ---------- t_1	$I_1\ I_2\ I_3\ I_4$ k
B.	$I_1\ I_2\ I_3$	Rx | ---------- t_3	$I_1\ I_2\ I_3\ I_4$ k
B.	$I_1\ I_2\ I_3$	Rx | ---------- t_5	$I_1\ I_2\ I_3\ I_4$ k
D.	$I_1\ I_2\ I_3$	Rx | ---------- t_7	$I_1\ I_2\ I_3\ I_4$ k
E.	V_1	---------- t_7	$I_1\ I_2\ I_3\ I_4$ k

I_i = infection with 50 L3/mouse/week (where i = 1,2,3,..)

V_1 = vaccination with 250 irradiated larvae/mouse

Rx = treatment of mice with ivermectin

t_i = time interval between treatment and second round of trickle infection (where i = 1, 3, 5 and 7 weeks)

k = all mice were killed for determination of worm burdens.

The mean worm burdens recovered from the drug-treated and vaccinated mice are shown and compared to their respective controls in Fig. 5.21. It can be seen that both chemotherapy and vaccination conferred resistance which lasted for up to 7 weeks (protection levels of >95%) in these low responder CBA/Ca mice despite continued re-infection.

5.10 Discussion

As previously mentioned in the introductory section, a monotonic rise in worm burdens to a stable level and a convex-age intensity or prevalence patterns are discernible in helminth infections in human communities, depending on the level of transmission and the interplay between immunity and exposure to infection (Anderson & May,1985 a,b).

In this chapter, emphasis was placed on the host genetic factors that may generate such epidemiological patterns. By employing a repeated infection experimental design in inbred laboratory mice, it has been possible to exclude differences in exposure to infection in the generation of age-convexity in parasite burden. The results must therefore be interpreted as evidence that acquired resistance plays an important role in determining the dynamic changes in parasite acquisition and loss in host populations. The strength of this argument lies in the fact that empirical findings have combined with a

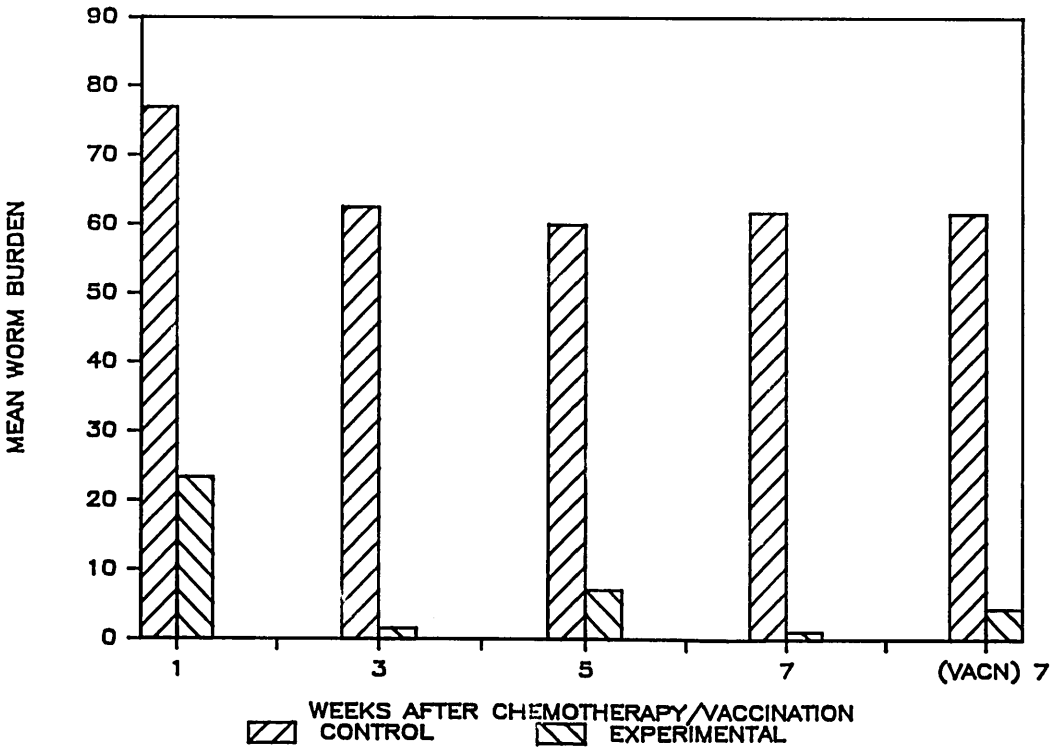


Figure 5.21. The duration of resistance to H.polygyrus in low responder mice. Resistance was induced by either repeated infection and chemotherapy or vaccination of mice with irradiated larvae.

The mean worm burdens from experimental mice are compared to control mice which had only the second round of trickle infections (5 mice per group). Note that mean worm burdens at t=1 week interval are significantly higher than at other periods ($P<0.001$, ANOVA on log (10) (x + 1) transformed mean worm burdens).

biologically realistic mathematical model that was able to generate, with only a change in the infection rate Λ, an increase in parasite burden with time in low responder mice and also in high responder mice subject to low infection doses. The model was able to predict a convex age-intensity pattern in the high responder mice exposed to a high infection rate. In nature, it is likely that susceptible individuals will be important in parasite transmission.

The application of both chemotherapy and vaccination in mice was able to generate a level of acquired resistance which was sustained at a high level for 7 weeks in low responder mice. The ability of vaccines to convert 'susceptible' individuals into the 'responder' class is encouraging in view of the current drive to develop vaccines for human helminth parasites.

Chapter 6

Serum antibody responses to and characterization of larval and adult antigens of *H.polygyrus*

6.1 Introduction

The population characteristics of acquired resistance to parasitic infections can in principle be readily quantified. These include the measurement of parasite burdens after infection; parasite reproductive stages (eggs or larvae); behavioural and morphological changes; parasite-induced host mortality, growth, or physiological status (Mitchell,1979; Wakelin,1985; Miller,1986).

The mechanisms of resistance, whether they be immunologically-based or not, are less easily characterized. Their dissection, however, forms a first step toward an understanding of immunity to parasitic infections (Barriga, 1981; Cohen & Sadun,1981; Wakelin,1984). Further, a better understanding of the mechanisms of immunity to parasitic infections at the molecular and cellular levels is desirable in the context of the development of prophylactic control methods (Cox,1978; Lloyd,1981) or the manipulation of immunoregulatory factors in the pathology of disease (Mitchell,1979; Roitt *et al*,1985). Unravelling the mechanisms of immunity to parasites helps to shed light on the biological relationships between host and parasite.

The quantitative measurement of the parameters relating to the expression of immunity such as antibody or cell-mediated effects and the demonstration of their parasite specificity and killing effect both *in vitro* and

in vivo is, therefore, of great interest at present. Having established a link between host responses and host protection, the parasite antigens which elicit such protection need to be characterized and their immunogenicity demonstrated. Subsequently, using modern molecular and biochemical techniques, purification and production of these antigens can be attempted (recombinant DNA technology) as an initial step in the production of effective vaccines (see Selkirk *et al*,1986).

The objectives of this chapter are, first, to review (in sections 6.2 and 6.5) the recent developments in the anlysis of the mechanisms of immunity to *H.polygyrus* in laboratory systems. Secondly, to provide a critical assessment of current research on antibody and cell-mediated immunity to *H.polygyrus* and attempts at the characterization of surface antigens of the parasite in order to identify 'gaps' in our knowledge of immunity to *H.polygyrus*. Results from experiments designed to contribute further to our present knowledge of serum antibody responses to *H.polygyrus* are presented in section 6.3 and characterization of surface antigens of *H.polygyrus* by radiolabelling, immunoprecipitation and SDS-PAGE techniques is reported in section 6.4. Specifically, immunological parameters in high and low responder mice subject to long-term repeated oral infection with *H.polygyrus* larvae are related to the dynamics of parasite acquisition and loss, as assessed by worm burdens in mice.

In section 6.5, the results are discussed in relation to the broader issues of genetic control of parasitic infections and their implications for community-based control of intestinal helminths.

6.2 Serum antibody responses

Immunity to H.polygyrus in mice is mediated by various cells in the presence or absence of antibody. Before discussing the specific role of antibody, therefore, mention of the role played by cellular immunity is necessary.

A role for macrophages in immunity to H.polygyrus has been reported (Chaicumpa, Jenkin & Fischer,1977; Chaicumpa & Jenkin,1978; Prowse, Ey & Jenkin,1978). Macrophages, in the presence of fresh immune serum, are able to adhere to and damage parasitic stages of H.polygyrus in vitro (Pentilla, Ey & Jenkin,1983), suggesting that only 'activated' macrophages are able to mediate immunity to H.polygyrus.

A marked serum eosinophilia has been described in H.polygyrus-infected mice (Ali, Behnke & Manger,1985; Cypess,1972; Jones,1974; Prowse et al,1978) and tissue eosinophilic infiltration has been reported (Liu, Cypess & Van Zandt,1974; Jones & Rubin,1974). A more direct in vitro role for eosinophils has been described by Hurley & Vadas (1983) by demonstration of eosinophil

adherence to infective larvae and their subsequent killing in the presence of antibody. The *in vivo* effect of eosinophils remains to be demonstrated (Pentilla, Ey, Lopez & Jenkin,1985). Schmitz *et al* (1982) has provided supportive evidence to show that the presence of activated oxygen species from 'metabolic burst' of granulocytes from rats and mice given a primary infection of *H.polygyrus* was responsible for host resistance. Interestingly, the demonstration of differential eosinophilia (using cyclophosphamide treatment and bone marrow chimaera techniques) in two inbred mouse strain- the high responder (to *H.polygyrus*) BALB/c and the low responder CBA/H mice- was correlated with heterogeneity in the acquisition of resistance (as assessed by worm burdens) in these two mouse strains (Hurley & Vadas,1983).

More attention has been focussed on the eosinophil rather than the neutrophil in cell-mediated resistance to parasitic helminths (Butterworth,1980; Kay,1985; Gleich & Loegering,1984), but, the role of the neutrophil in resistance to *H.polygyrus* has recently been emphasized (Pentilla *et al*,1985). Demonstration of neutrophil-mediated immunity *in vivo* in the presence of antibody (Pentilla, Ey & Jenkin,1984a) and the reduced infectivity of L3 cultured *in vitro* in the presence of neutrophil and eosinophil-rich immune serum (not normal serum) have been reported (Pentilla, Ey & Jenkin,1983; Pentilla, Ey & Jenkin,1984b). Reduced infectivity of L3 is thought to be mediated by antibody in the presence of complement via

the alternative pathway of activation (Prowse, Ey & Jenkin, 1979). Taken as a whole, these results suggest that only activated macrophages and 'altered neutrophils' are able to effect host protection (Pentilla et al,1985). It has also been suggested that neutrophils play an important role in early immunity to H.polygyrus in mice via studies of the resistance induced following the in vivo selective depletion of neutrophils with rat monoclonal antibodies specific for mouse granulocytes (Pentilla et al,1985).

Hypothymic (nude) mice have also been used to demonstrate the role of T-cellular immunity to H.polygyrus (Mitchell,1979). It has been observed that, following multiple infections, nu/nu mice would not expel their worms whereas their F1 littermates (nu/+) did expel their worms (Prowse, Mitchell, Ey & Jenkin,1978). Transfer of immune mesenteric lymph node cell (IMLNC) to these mice restored their ability to produce IgG antibody but was not translated into increased resistance as assessed by worm burdens (Prowse et al,1978).

Subpopulations of thymus-derived cells play an important part in immune regulation (Roitt et al,1985; Hood et al, 1984). For example, mice subject to the anti-T-suppressor cell agent, 2'-deoxyguanosine, had their immune responses to H.polygyrus restored but the immunity elicited was not host protective since worm burdens in mice were not significantly reduced (Pritchard, Ali & Behnke,1984).

The observed increase in IL-2 secretions (the T-cell growth stimulating factor) from T-cells of mice infected with H.polygyrus (Prowse,1982) further supports the involvement of T-cells in immunity to this parasite. Preliminary studies using FACS (Flourescent activated cell sorter) techniques suggest that in low responder CBA mice infected with H.polygyrus, there was an increase in T-cellular activity in the lymphoid tissues of these mice and that the ratio of cell surface marker phenotypes suggested a balance in favour of T-helper cells (Parker & Inchley,1985).

More generally, it has been shown that antibody responses in mice during infection with a variety of parasites, including H.polygyrus, are markedly T-cell dependent (Mitchell,1979; Mitchell, Anders, Brown, Handman, Roberts-Thompson, Chapman, Forsyth, Kahe & Cruise,1982). Further studies are obviously needed to test the effect of chemical agents known to alter the T-cell component of the immune response e.g. anti-Thy antiserum, or the use of mouse strains with specific genetic defects in cell-mediated immunity. In addition, the use of the recently available T-cellular assay systems would greatly contribute to our present knowledge on the mechanisms of immunity to chronic infections such as H.polygyrus.

The role for antibody in the expression of immunity to H.polygyrus has also been assessed. Jenkins & Carrington, (1981) showed that Biozzi mice with known high and low

antibody responses to a variety of antigens, but equivalent cell-mediated and macrophage activity (Biozzi et al,1979), responded differently to multiple infections with H.polygyrus. The high responder mice were significantly more resistant i.e. had fewer worms, than low responder mice, suggesting that resistance to this parasite was mediated by antibody (Jenkins & Carrington,1981).

Additional support for the role of antibody in resistance to H.polygyrus has come from the successful passive transfer of immunity by immune serum to naive recipients, although results have sometimes been contradictory (Jones & Rubin,1974; Bartlett & Ball,1974). Mice given immune serum expelled adult H.polygyrus (Behnke & Parish,1979) and transfer of immunity was shown to involve the co-operation between lymphoid cells and antibodies (Behnke & Parish,1981). The genetics and sex of the donor host - female mice being more efficient at transfer than males- influence the degree to which immunity could be passively transferred (Dobson & Owen, 1978; Dobson, Brindley & Sitepu,1982). The quantity and quality of the serum (Dobson,1982) and the timing of serum adminstration (Behnke & Parish,1979) are also important. One postulated mechanism for the effect of transferred immune serum is the ability of the serum to kill and reduce the infectivity of larvae (Dobson & Cayzer,1982). However, larvae incubated in immune serum were protected from the effects of passively transferred serum as judged by the stunting of adult worms and altered

sex ratios (Dobson & Cayzer,1982). Another mechanism would be as envisaged in the two-step process of parasite attrition already documented for Nippostrongylus brasiliensis (Ogilvie & Love,1974) and Trichuris muris (Wakelin,1975), where antibody binds to and damages the worms, and then cellular responses effect worm expulsion.

Conversely, there is evidence for the failure of passively transferred serum to induce immunity in recipients (Chaicumpa, Jenkin & Rowley,1976; Cypess,1970; Panter, 1969). Such failure could be due to the use of primary infection serum (Williams & Behnke,1983), insufficient and low quality serum (Dobson,1982) and immunosuppressive worm products (Dobson & Cayzer,1982; Pritchard & Behnke, 1985).

Having demonstrated the host protective effect of passively transferred serum, it is of interest to isolate the specific serum components and then to correlate their presence with host protection (Williams & Behnke,1983).

Serum immunoglobulin level, as assessed by either single radial immunodiffusion or radioimmunoassay, in mice given multiple infections were observed to increase within 2 weeks of challenge infection. IgG1 is the predominant isotype (Molinari, Ebersole & Cypess,1978; Crandall, Crandall & Franco,1974; Prowse, Ey & Jenkin,1978; Prowse, Mitchell, Ey & Jenkin,1978; Chapman, Knopf, Anders & Mitchell,1979; Prowse, Mitchell, Ey & Jenkin,1979;

Pritchard, Williams, Behnke & Lee,1983; Williams & Behnke, 1983; Pritchard, Behnke & Williams,1984) and IgG1 hypergammaglobulinaemia, especially during high dose chronic primary infections, is believed to be a potential 'blocking antibody' rather than host protective (Mitchell et al,1977; 1982; Chapman et al, 1979).

On the other hand, there is evidence that purified IgG1 from immune serum obtained from mice subject to multiple infections accounted for the greater proportion of host protection (Pritchard et al,1983), but IgG1 from primary infection serum was not found to block host protection (Pritchard et al,1984).

It is thought that this IgG1 hypergammaglobulinaemia is a result of T-dependent B^{γ}-cell stimulation by high dose chronic antigen exposure (Prowse et al,1978; Chapman et al,1979a) since heterologous antigen (SRBC) can induce a similar effect (Chapman et al,1979b). As most of this IgG1 was total rather than parasite specific, it has been suggested that its enhanced production probably reflects an imbalance in the concentration of IgG2a (Chapman et al, 1979; Mitchell et al,1982). However, increased catabolism of IgG1 in infected mice could be one mechanism whereby the parasite evades the immune response (Brown, Crandall & Crandall,1976; Chapman et al,1979b).

It has been shown that a substantial part of this IgG1 is parasite specific (Jacobson, Brooks & Cypess,1982) ranging from 25% (Cypess, Ebersole & Molinari,1977), 45% (Molinari, Ebersole & Cypess, 1978) to 48% (Pritchard et al,1983) of total serum IgG. The method was the quantitative antibody-binding method of Nash & Heremans (1969), a procedure based upon the determination of immunoglobulin concentration before and after adsorption by optimally concentrated H.polygyrus antigen. Serum IgA, IgM and IgG2a appear not to reveal any significant increase during infection nor could they be correlated with the appearance of host-protective antibodies (Williams & Behnke,1983).

Intestinal perfusates of multiply infected mice showed an increase in IgG1 and IgA, although it was not immediately clear whether this increase was due to local antibody or leaked plasma proteins (Cypess, Ebersole & Molinari,1977). Local antibody or cell production is likely to be important in lumen-dwelling parasites where antigen homing ought to be effectively achieved (Befus & Bienestock,1979).

Inbred mouse strains of the NIH and CBA/Ca genotypes when subject to long-term repeated low-level infections with H.polygyrus show differential patterns of worm acquisition and loss (see chapter 5). NIH mice are of the 'high responder' phenotype and CBA/Ca are 'low responders'. The question arises, therefore, whether

this differential resistance, manifest as low and high worm burdens in NIH and CBA/Ca, respectively, correlates with the ability of these two mouse strains to produce protective antibodies. It also bears on the problem of whether quantitation of antibody can be used to assess the intensity of parasitic infections of man in endemic areas. Experiments are described in which sera from individual mice were collected and analysed by ELISA, immunoprecipitation, SDS-PAGE and autoradiography techniques.

6.3 Results from ELISA

6.3.1 Antibody responses in low responder CBA/Ca mice

Serum antibody responses in low responder CBA/Ca mice during repeated, weekly, infection at three dose levels for 12 weeks to optimally concentrated crude adult H.polygyrus antigen are shown in Fig. 6.1. The two graphs, (a) and (b), show anti-H.polygyrus antibodies of the IgG1 and IgM classes, respectively. Antibodies of either class were significantly elevated at all doses of infection compared to normal mouse sera (NMS). The anti-H.polygyrus antibodies in mice exposed to the low (10L3/mouse/week) and medium (25L3/mouse/week) doses of infection were, however, similar (Fig. 6.1). At the high dose of infection (50L3/mouse/week), specific anti-parasite IgG1 antibodies rose rapidly within 2 weeks after the initial infection

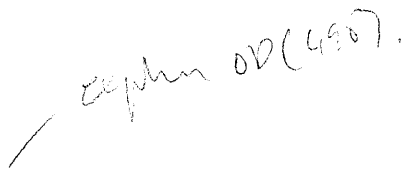
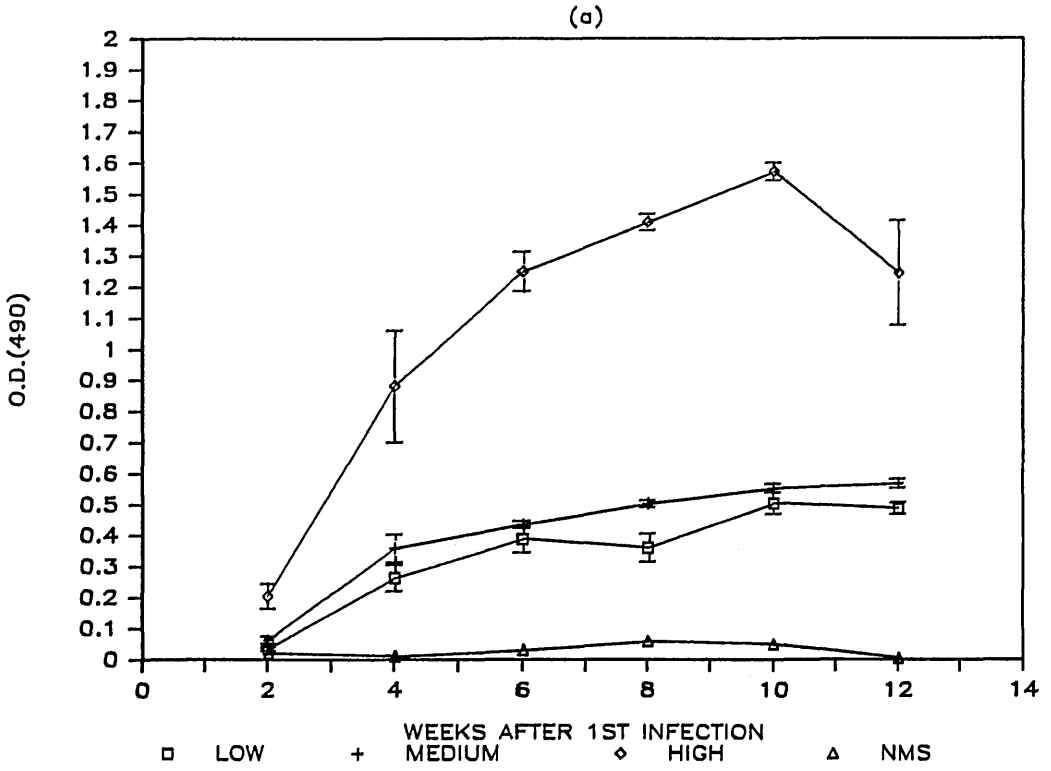
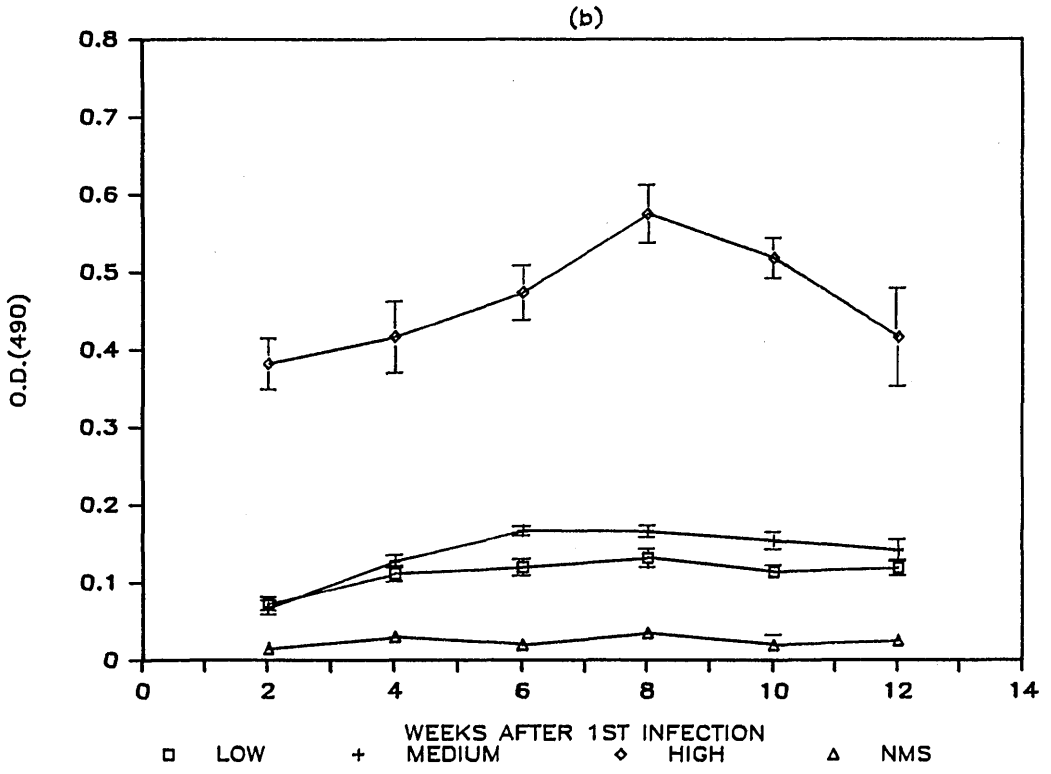


Figure 6.1. Serum antibodies of the IgG1 (a) and IgM (b) isotypes in low responder CBA/Ca mice exposed to H.polygyrus at three levels of infection during a 12-week period.

Squares - low dose of infection (10L3/mouse/week);
Crosses - medium dose of infection(25L3/mouse/wk);
Diamonds- high dose of infection (50L3/mouse/wk);
Triangles- Normal mouse serum (NMS)(=control).
The vertical bars represent the standard error of the mean (n=10 mice per group).


(a)
O.D.(490)
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
WEEKS AFTER 1ST INFECTION
LOW
MEDIUM
HIGH
NMS


(b)
O.D.(490)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
WEEKS AFTER 1ST INFECTION
LOW
MEDIUM
HIGH
NMS

and remained stable from 6 weeks onwards (Fig. 6.1a), whereas IgM antibodies remained elevated but stable during the infection period of 12 weeks (Fig. 6.1b).

6.3.2 Comparative antibody responses in low (CBA/Ca) and high (NIH) responder inbred mice

Serum anti-*H.polygyrus* antibodies from low and high responder mice exposed to a high infection dose of 50L3 per mouse per week are compared in Fig. (6.2). In Fig. 6.2(a), antibodies of the IgG1 isotype were significantly elevated in the two mouse strains, but antibody level in the low responder CBA mouse strain were much higher than those in the high responder NIH mice throughout the experimental period. The IgM antibody responses did not appear to differ between the two mouse strains.

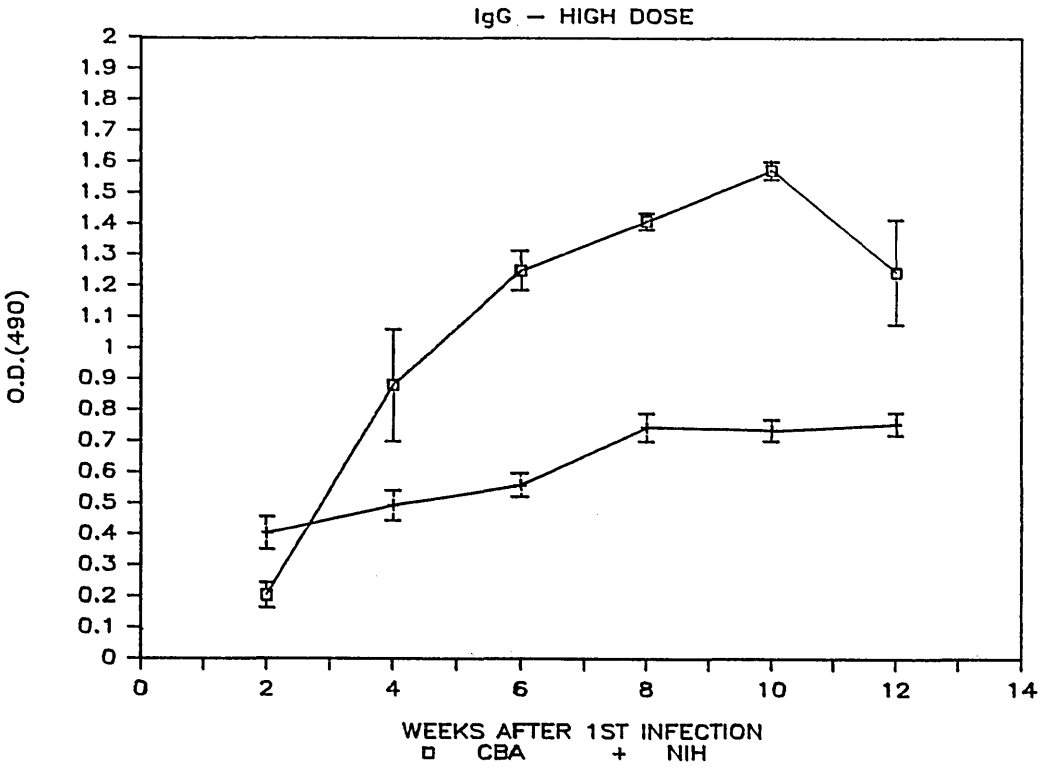
The mean number of worms recovered from the low and high responder mouse strains during repeated infection are also compared in Fig. 6.3. Worm burdens were higher in the low responder mice than in the high responder mice during the experimental period.

Figure 6.2. Comparison of serum antibodies to adult H.polygyrus in low (CBA/Ca) and high (NIH) responder mice repeatedly exposed to infection at a high dose of 50L3 per mouse per week for 12 weeks.

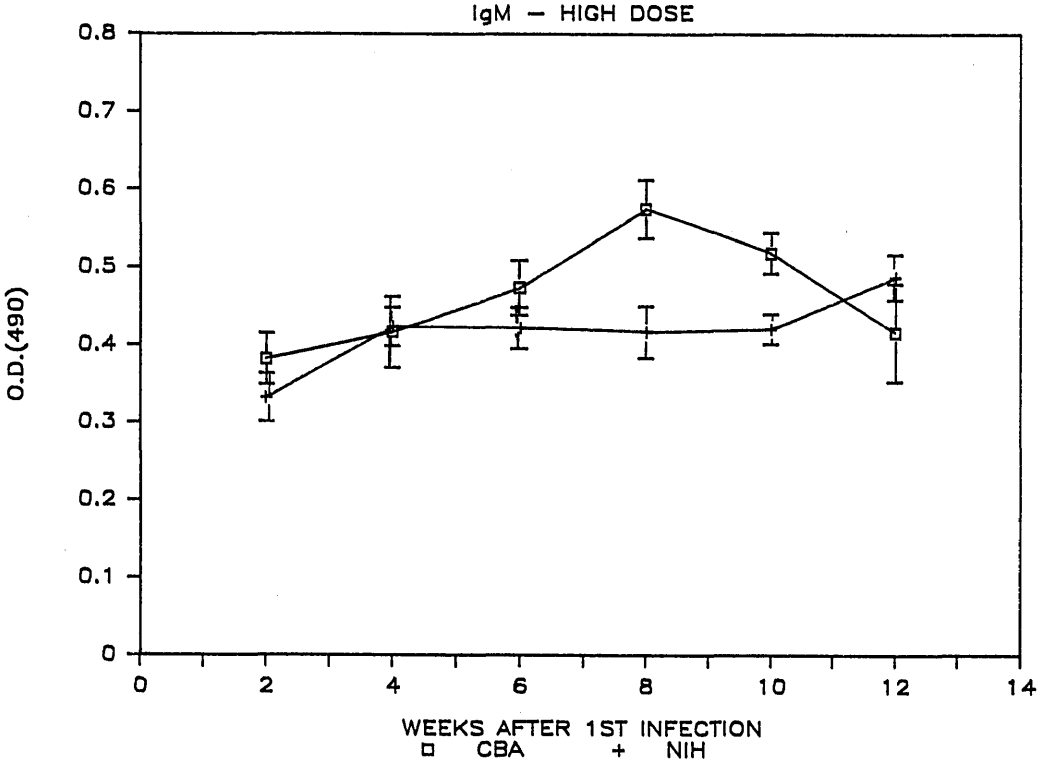
(a) showing levels of IgG1 antibodies and

(b) IgM antibodies.

(a)


IgG — HIGH DOSE
O.D.(490)
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
WEEKS AFTER 1ST INFECTION
□ CBA + NIH

(b)


IgM — HIGH DOSE
O.D.(490)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
WEEKS AFTER 1ST INFECTION
□ CBA + NIH

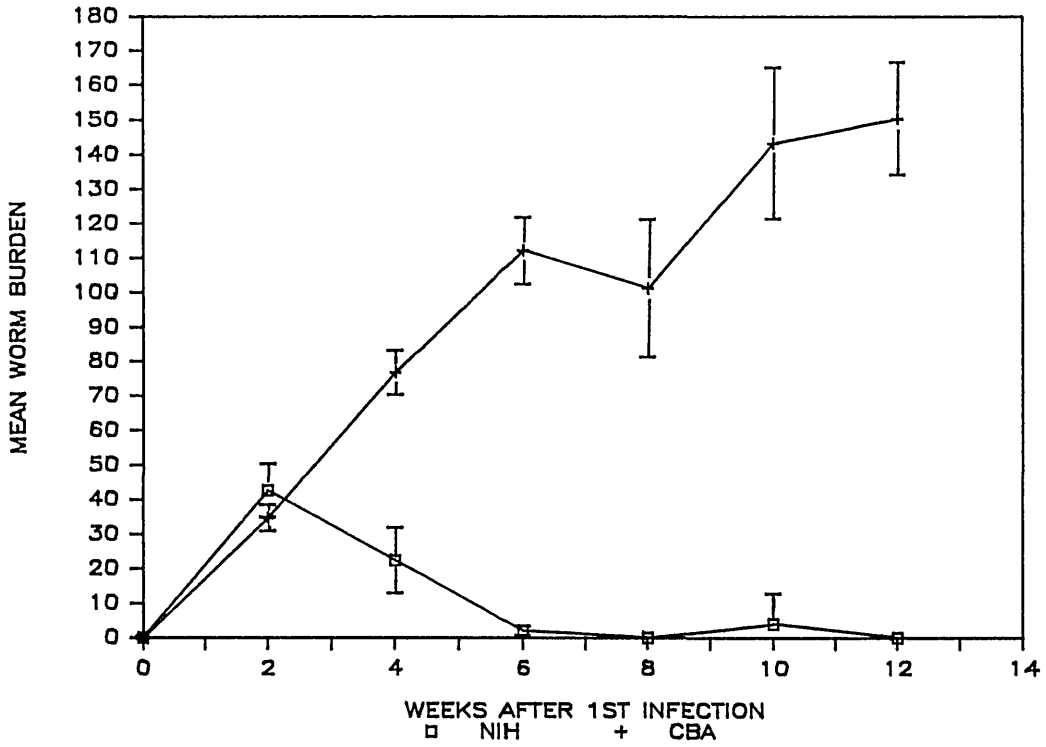


Figure 6.3. The mean worm burdens in low(CBA/Ca) and high (NIH) responder inbred mice during repeated infection with 50 L3 H.polygyrus/mouse/week.

Squares - observed points (n = 5 NIH mice);

Crosses - observed points (n = 10 CBA/Ca mice);

Vertical bars - standard error of the mean.

6.4 Characterization of H.polygyrus antigens

Despite the conventional 'text book' perception of the exo-surface of parasitic nematodes as biologically inert and serving only to provide physical protection, information now available shows that the nematode cuticle is complex, consisting of a dynamic outer hypodermal membrane with amino acid-derived collagenous material (Lee & Aitkinson,1976). The cuticle is physiologically active (Lee,1972) and antigenically dynamic (Philipp et al, 1980; Ogilvie et al,1980; Pritchard et al,1986).

Surface antigens, by virtue of their location, and in vitro released products (IVR) also known as excretory-secretory (ES) products, have recently received a great deal of attention as probes for the detection of immunity (Maizels et al,1982). Their potential role in immuno-diagnosis, as target molecules in the study of parasite survival strategies and in providing valuable information on the function and structural conservation of nematodes is clearly important (Philipp et al,1980; Parkhouse et al, 1981; Philipp & Rumjanek,1984).

The biochemical and molecular approach to the study of these antigens has recently gained impetus. Surface antigen may be labelled with radioactive material and/or then extracted by homogenization or sonication, or labelled after release into incubating medium (Parkhouse et al,1981). Also, surface antigens may be selectively stripped off the surface by use of ionic detergents (Pritchard et al,1986;

Grencis et al,1986). Following solubilization, labelled antigens may be analysed by gel electrophoresis and their immunogenicity confirmed by immunoprecipitation or immunoblotting with reactive sera. Thus, it is possible to identify sets of antigens, and whether or not these antigens are species or stage specific, and to determine the extent of cross-reactivity with homologous or heterologous antigens. Translation products can be synthesized in vitro by mRNA isolated from these parasite products and tested for their immunogenicity (Carr & Pritchard,1986; Selkirk et al,1986).

Using these techniques, several nematode parasites have been shown to possess a restricted set of antigens. These include N.brasiliensis (Maizels et al,1982); Trichinella spiralis (Philipp et al,1980;1981; Parkhouse et al,1981); Brugia (Sutanto et al,1985; Maizels et al, 1983; 1985; Parkhouse et al,1985; Philipp et al,1986); Onchocerca (Ouissi et al,1981; Philipp et al,1984) and more recently, Wuchereria bancrofti (Maizels et al,1986; Morgan et al,1986), the hookworms, Necator americanus (Carr & Pritchard,1986) and Ancylostoma caninum (Hotez et al,1985); Ascaris spp (Kennedy et al,1986) and Toxocara canis (Meghji & Maizels,1986).

Stage-specific antigens of the mouse hookworm H.polygyrus have recently been analysed by SDS-PAGE (Pritchard et al,1984; Pritchard et al,1986). The tissue L4 stage expressed immunogenic antigens with an apparent

molecular weight of 16 kDa and the adult antigen resolved at 60-70 kDa as revealed by immunoprecipitation with immune mouse serum (Pritchard, Maizels, Behnke & Appleby, 1984). It has been suggested that the apparent differences in the expression of surface antigens during the maturation of the parasite is a candidate mechanism of parasite evasion of host immune responses (Pritchard et al, 1984). Here, the results from similar biochemical and molecular analyses of surface antigens of H.polygyrus in low responder (CBA/Ca) and high responder (NIH) mice during long-term trickle infections are reported.

6.5 Results from SDS-PAGE and autoradiography

6.5.1 Radiolabelling and immunoprecipitation

Iodogen labelling has been preferentially used over the Bolton-Hunter or Chloramine-T methods due to its apparently limited penetration of the cuticle (Selkirk et al,1986). Antingens labelled with I^{125} were solubilized using 1% deoxycholate and further reduced with 5% 2-mercaptoethanol after immunoprecipitation with immune serum.

Labelled antigens were routinely counted on a gamma counter during preparation for SDS-PAGE. Table 6.1 shows the labelling efficiency of the method for H.polygyrus surface antigens. Adult worm antigens were poorly TCA precipitable, implying that there were few peptide-

Table 6.1. The efficiency of ^{125}I-labelling of surface antigens (Iodogen method) of 6-day old fourth-stage larvae (D6L4) and adult H.polygyrus and the percent TCA-precipitable supernates*.

(Data in counts per minute (cpm)/µl)

	cpm/µl	% TCA-precipitable
D6L4	2900	40
Adult	3500	20

* Following immunoprecipitation with trickle infection sera, the average cpm for deoxycholate reduced soluble preparation was 2640 cpm/µl and 19080 cpm/µl for the adult and larval surface antigens, respectively.

containing components that were labelled, but larval material was satisfactorily labelled (=40%). Thus, solubilized immunoprecipitates of adults had less radioactivity than larval material and the adult material was not easily identified by autoradiography even after 2 weeks of exposure of the film.

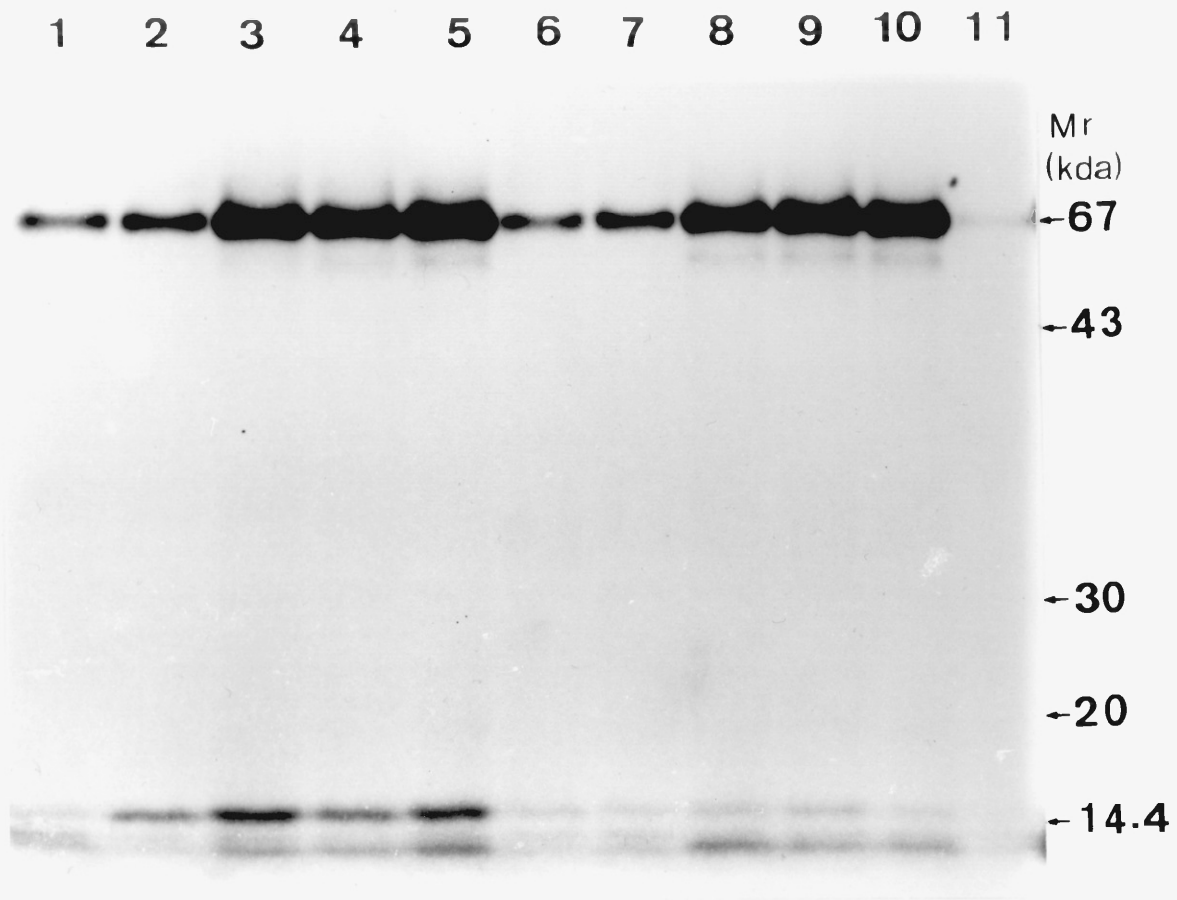
6.5.2 SDS-PAGE and autoradiography

Immune mouse serum from high and low responder mice showed reactivity to a restricted number of bands when resolved by SDS-PAGE (Plate 6.1). Two major components found under reducing conditions ran at apparent molecular weights (M_r), 16 kDa and 65-67 kDa in preparations from both mouse strains during the 12-week repeated infection period. In both mouse strains, however, qualitative differences are observed in the changes in intensity of the bands with time. In lanes 1,2,6 and 7 (representing 2-4 weeks after the first infection), the bands were less intense than in lanes 3-5 and 8-10 (representing 6-12 weeks after first infection). Normal mouse serum (lane 11) resolved only faintly at M_r 65 kDa, suggesting that this band could include host albumin, notwithstanding elaborate efforts to perform the analysis under reducing conditions.

Plate 6.1. An autoradiograph of SDS-PAGE analysed immunoprecipitates (under reducing conditions) manufactured between ^{125}I-labelled (Iodogen) surface antigens of 6-day old larval H.polygyrus and serum from high and low responder mice repeatedly exposed to infection with 50 L3/mouse/week.

Pooled sera were obtained from low responder CBA/Ca mice (lanes 1-5) and high responder NIH mice (lanes 6-10) at 2 weeks (lanes 1,6), 4 weeks (lanes 2,7), 6 weeks (lanes 3,8), 8 weeks (lanes 4,9) and 12 weeks (lanes 5,10) post-initial exposure. Normal mouse serum is shown in lane 11. Molecular weight markers (Mr) are shown as arrows.

PLATE 1



6.6 Discussion

The experiments have attempted to show, for the first time, the correlation between parasitological and immunological parameters in inbred mouse strains that respond differently to long-term trickle infection with H.polygyrus. The mean worm burdens in these mice were similar until 2 weeks following the first infection, when NIH high responder mice lost almost all their worms despite continued infection. In marked contrast, the low responder CBA/Ca mice continued to gain worms throughout the experimental period (Fig. 6.3). These results are interpreted as evidence for heterogeneity in the ability of these two mouse genotypes to acquire resistance to infection with this parasite (see also chapter 5).

Serum IgG1 antibodies (with or without immune lymphoid cells) have been shown to be host protective as judged by the reduced number and size of worms in mice given or producing elevated levels of this isotype (Williams & Behnke,1983; Pritchard, Williams, Behnke & Lee,1983; Pritchard, Behnke & Williams,1984). Much debate has centered on the ability of IgG1 antibodies to mediate host resistance since it has also been suggested that high levels of this particular isotype are counterproductive to host resistance i.e. IgG1 is 'blocking' antibody (Chapman et al,1979b; Mitchell,1979; Dobson & Cayzer,1982).

The results in Fig. 6.1 indicate that low responder mice have the ability to produce IgG1 anti-parasite antibodies in a dose-dependent manner. In fact, relative IgG1 antibody levels were more elevated in low than in high responder mice (Fig. 6.2). These results add support to the hypothesis that high levels of IgG do not imply protection against infection. The presence of high anti-parasite, host-protective antibody levels in these 'low' responder mice is puzzling. This result questions the reliability of serum antibody levels to accurately reflect the immune status of the host in epidemiological or ecological studies (cf. Voller & De Savigny,1981). The interpretation of seroepidemiological data for intestinal nematodes is likely to present problems due to the difficulty in distinguishing between classes of the population who have just been infected, lost their infection or are immune to infection.

Anti-parasite antibodies are obviously not the only decisive factor in determining the pattern of worm burden in low or high responder mice. It has been postulated that survival of H.polygyrus in mice producing elevated IgG1 antibodies could be due to increased T-suppressor cell activity (Pritchard et al,1983). Further work on the celluar aspects of immunity to H.polygyrus is needed.

Mouse genotype clearly influences the level of acquired resistance in mice,since NIH and CBA/Ca mice exposed to the same high dose infection revealed widely differing patterns of worm burdens and anti-parasite IgG antibodies. At the 2-week time point of the trickle infection, NIH mice had higher antibody levels than CBA/Ca mice, and thereafter, the situation was reversed (Fig. 6.2a). The possibility, therefore, that this 2-week period could represent a 'window' through which the mouse genotype determines whether the mouse strain becomes a 'low' or 'high' responder is worthy of investigation. (Mitchell,1979; Wakelin,1985a).

Additional evidence from SDS-PAGE analysis of immunoprecipitates manufactured between mouse sera during trickle infection, and surface antigens of the larval parasite (Plate 6.1) gives the overall impression that the NIH and CBA/Ca mouse strains do not differ greatly in their ability to 'recognize' surface antigens of H.polygyrus. Similar results were obtained from low responder C3H and high responder Balb/c mice under different conditions of exposure to the ones described here (Maizels, unpublished observations). The results do support Pritchard, Maizels, Behnke & Appleby's(1984) observation that the 16 kDa and 67 kDa components are immunogenic. Additional experiments would benefit from further immunochemical analysis of the properties of these components. Recently, the ability of the cationic detergent, cetyltrimethylammonium bromide (CTAB) to

selectively elute surface antigens of the nematodes H.polygyrus, T.spiralis and N.americanus in reasonable quantities and acceptable purity (Pritchard, Crawford, Duce & Behnke,1985), has given impetus to their possible use as immunogens in vivo. Mice injected with 50 µg of CTAB-eluted antigen in FCA were significantly protected from challenge infection with T.spiralis (Grencis, Crawford, Pritchard, Behnke & Wakelin,1986).

The antibody response to stage-specific, surface antigens of Trichinella spiralis has been compared between strong responder (NIH) and weak responder (C3H) inbred mouse strains (Jungery & Ogilvie,1982). In this study, temporal differences in the ability of primary infection serum from the two mouse strains to mediate eosinophil adherence to, and to immunoprecipitate ^{125}I-labelled, stage-specific cuticular antigens of the parasite, were observed. This differential recognition of antigens in the two mouse strains has been linked to the genetic control of the rejection of T.spiralis from the mice (Jungery & Ogilvie,1982). It would be interesting to find out whether similar results could be obtained from mice during repeated exposure to infection.

The biochemical and immunological approach to the study of the antigens of the major hookworm and other gastro-intestinal nematode parasites e.g. N. americanus and A. lumbricoides (Carr & Pritchard,1986; Kennedy, Qureshi, Haswell-Elkins & Elkins,1986), is likely to yield additional information on immunity to these parasites

in man. A 33 kDa component of ES antigens of N.americanus was recognized by sera of human subjects infected with hookworm (Carr & Pritchard,1986). This 33 kDa component is a candidate for immunodiagnosis of N. americanus infections since sera from individuals infected with Ascaris and Trichuris would not cross-react with it (Carr & Pritchard,1986). These, and other findings, greatly improve the prospects for immunodiagnosis of intestinal parasites (Kennedy et al,1986; Carr & Pritchard, 1986) since whole worm antigens are poor sources of functionally important antigens and hence, poor immuno-diagnostic tools (Ogilvie & De Savigny,1982).

Chapter 7

Summary discussion

This final chapter attempts a synthesis of the major findings of this project. The major results are discussed in light of the population dynamics of host-gastrointestinal nematode infections. The gaps in our knowledge about host-helminth population interactions are highlighted and possible directions for future research in this area are discussed.

Genetic control of parasitic infections by hosts has recently received wide attention (Wakelin,1985a). The primary and original impetus for this interest lies in the potential for host genetic manipulation to produce animals that are economically beneficial to the needs of man (i.e. to breed disease resistant animals)(Dargie,1982; Albers & Gray,1986). Moreover, the implications of genetic control of parasitism to the control of parasites by chemotherapy or vaccination have recently been emphasized (Anderson & May,1982; Anderson & May,1985 b,c). Genetic control of parasitic infections in man is difficult to demonstrate, given the difficulties in experimentation with man or higher animals and the paucity of information on the association between genetic loci such as the HLA complex and parasitic diseases. Consequently, laboratory studies using mice have proved useful in the analysis of the genetic factors responsible for the interaction between hosts and parasites.

Experiments have been described in which a heterogeneous outbred mouse strain (MF1) was selected for 'high' and 'low' parasite egg counts following a standard infection, over four generations. Heritability of the trait 'faecal parasite egg counts' was shown to be quantitative rather than qualitative (see Brindley, He, Sitepu, Pattie & Dobson,1986), with an estimated heritability score of approximately 0.2 per generation. This observation suggests that future work must aim at selecting the host over 10 or more generations in order to gain more information on the degree of heritability of this trait. Another possible approach would be to cross the high and low inbred responder mice (chapter 5) and then back select the mice from the F1 generation. The results reported in this thesis tentatively suggest that innate resistance to H.polygyrus in mice is genetically controlled and that outbred mice consist of a genetically heterogeneous population, with respect to their response to H.polygyrus infection.

The basic population parameters controlling the flow of H.polygyrus in mice were estimated under controlled laboratory conditions. The potential of the host to regulate the parasite population is linked to many rate-determining population parameters. In particular, the presence or absence of density-dependence in the parameters will determine the degree to which the host can regulate the growth of the parasite population (Anderson, 1976).

Both density- and age-dependent fecundity of H.polygyrus were demonstrated in laboratory mice (chapter 4). The significance of density-dependence in the regulation of parasite populations in natural host communities should be considered in conjunction with the adverse environmental conditions and other density-independent ecological factors (e.g. seasonality in transmission) affecting the transmission of parasites.

Laboratory-based studies can also help to enhance our understanding of more complex natural communities. It often helps to make simplifying assumptions in attempts to dissect the components that determine observed patterns. Laboratory studies can be performed under rigorously controlled conditions, such that it is possible to investigate several factors one at a time. One very obvious criticism of laboratory experiments is that the findings may not be analogous to the real world. However, provided the results are interpreted sensibly, laboratory studies can provide powerful tools with which to generate discussion and suggest productive areas for field research. With this in mind, experiments in which mice of high and low responder phenotypes were repeatedly exposed to H.polygyrus at varying levels of infection, were conducted in order to mimic the epidemiology of intestinal nematodes (e.g. hookworm) in areas endemic for these parasites. The choice of mouse strains was made in order to gain more insight into the influence of host genetics on the dynamics of helminth transmission. Mathematical models

provide an additional set of analytical tools with which to sharpen our understanding of the host-parasite relationships (Anderson,1986).

Using this experimental approach, distinct patterns were generated in low and high responder mice, depending on the mouse strain and the level of exposure to infection. In low responder mice mean worm burdens increased monotonically at all doses of infection in the first 6 weeks of infection, but appeared to decrease slowly towards the end of the experiment at the lower doses of infection (Fig. 5.1). The immigration-death model, with the assumption that host immunity did not influence parasite population growth, was able to mimic the general pattern of change in worm burdens/host through time. Careful examination of the data suggested that whereas mice exposed to the low infection doses appeared to loose their infection as the experiment progressed, the mice exposed to the high dose of infection continued to gain more parasites. This result has been interpreted as evidence of high-dose host immunosuppression (Behnke et al,1983; Dobson et al,1985). Switching the dose of infection during trickle infections and superimposing the effects of protein malnutrition (Slater & Keymer,1986b) on the host genotype may yield more information on this aspect of host immunity to H.polygyrus.

The presence of putatively host-protective, parasite-specific antibodies (IgG1) in large quantities in these low responder mice (Fig. 6.2) suggests that perhaps the mechanism of host protection to H.polygyrus in mice is at the level of mucosal responses in combination with cellular immunity as opposed to serum-antibody mediated. It is hoped that future work will attempt to understand this intriguing phenomenon. The fact that the observed increase in worm burdens in CBA/Ca mice (host immunosuppression) is not apparent in NIH mice testifies to the importance of host genetics in determining the dynamics of worm acquisition and loss (Fig. 5.1). In NIH mice (high responders), convex age-intensity and prevalence patterns were generated at the high dose of infection (Fig. 5.13). As the period of repeated infection increased, mean worm burdens became higher in mice exposed to the low rather than the high dose of infection, a theme captured satisfactorily by the mathematical model incorporating increased parasite death rate and reduced parasite establishment as a function of past exposure of infection. A major conclusion to be drawn from these results is that acquired immunity in hosts can generate convexity in changes of parasite burdens with host age. However, the role of changes in exposure to infection with age in generating similar patterns in man cannot be excluded at present.

Berding, Murray, Slater & Keymer(1986) have successfully employed a mathematical approach to the analysis of changes in acquired immunity in mice as a function of host nutritional factors. The main difference between their model and the one employed here lies in the way in which host immunity is believed to operate on intestinal parasites. Berding et al(1986) have assumed a threshold effect of immunity on the mortality of parasites whereas the model based on the Anderson & May (1985b) framework described in this study assumes a linear relationship between the summed experience of past infection and both reduced parasite establishment and increased parasite death rate. Future generations of mathematical models could incorporate more complex non-linear relationships between parasite establishment and/or death rate and past experience of infection. However, further experimental work on quantifying the precise mode of action of host immunity on parameters such as parasite establishment, mortality and fecundity is clearly needed.

Taken together with the results described in chapter 6, the elevated parasite-specific antibodies in the two mouse strains, compared to naive mice, augur well for the sensitivity of serodiagnosis to detect nematode infections. The applicability of the technique will ultimately depend on the degree of cross-reactivity with other helminth infections (Selkirk et al,1986; Denham et al,1986; Smithers,1986; Philipp & Rumjanek,1984).

The results are somewhat disconcerting in that the relationship between parasite burden and host immune status may not be obvious from quantitative comparisons of antibody titres and parasite counts. The similarity in the ability of high and low responder mouse sera to detect accessible surface components of the parasite (Plate 6.1) also poses several questions worthy of investigation. For example, is it the genetic capacity of the host and/or the evasive mechanisms evolved by the parasite, which ultimately determine the course of infection in individual mice?

The enhancement of long-lasting resistance (Fig. 5.21) in low responder mice through either chemotherapy (Fig. 5.15) or vaccination with irradiated larvae (Fig. 5.18) suggests that the removal of adult worms by drug treatment and the presentation of antigen to the host during the initial stages of infection is crucial to the development of acquired resistance. Defective presentation of antigens such as anti-ring-necked pheasant egg white lysozyme and anti-hen egg white lysozyme, by macrophages from Biozzi low antibody responder mice has been reported (Adorini & Doria,1981).

Predisposition to helminth infections in man (Anderson,1986) may have a genetic basis, although it is more likely that genetic, behavioural and nutritional factors interact to create the observed patterns of helminth parasite intensities in human and animal communities. From the point of view of community control

of helminth infections, the elucidation of predisposition may be academic as, regardless of the factors that generate it, once identified, treatment can be focussed on these individuals or groups of individuals predisposed to heavy infection (Anderson & Medley,1985; Anderson,1986; Haswell-Elkins, Elkins & Anderson,1986). The control of helminth infections in a given area will also depend on the available financial resources, improved hygiene, better sanitation and nutrition, widespread education, reduced poverty and better standards of living. The ultimate test of insights generated by laboratory and theoretical work into the population dynamics of parasitic infections (such as expounded in this project) rests in the field. It seems clear, however, that an improved understanding of the population ecology of parasite transmission and parasite population regulation should ultimately help to improve the design of effective measures to control helminth infections in man.

SUMMARY

The main findings of this study may be summarised as follows:

1) A significant linear relationship between the number of female H.polygyrus and eggs/female/16h was found in outbred MF1 mice given a primary infection.

2) The frequency distribution of the proportion of outbred MF1 mice excreting high and low parasite eggs during selection was found to be asymmetric by the fourth generation of inbreeding.

3) The estimated heritability of the trait 'faecal parasite eggs/16h' in MF1 mice was 0.17 ± 0.08. The realized heritability estimated from the regression of the response to selection and the cumulative selection differential over 4 generations was 0.21 ± 0.29 in the 'high' line and 0.25 ± 0.04 in the 'low' line.

4) A significant negative phenotypic correlation between parasite eggs/mouse/16h and mouse litter size was found in generation II mice, but was not observed in generations III and IV.

5) The rate of parasite establishment in both inbred and outbred mice given varying single doses of H.polygyrus was found to be linearly dependent on

the dose of infection in the range 10 to 800 H.polygyrus per mouse. On average, 44% of the worms established in outbred MF1; 66% established in inbred CBA/Ca and 57% established in inbred NIH mice.

6) The sex-ratio of adult H.polygyrus was found to differ significantly from a 1:1 ratio, especially in mice given a high dose of infection. More female than male worms were recovered during a single infection of 12 weeks duration in inbred mice but the pattern was reversed in outbred mice over a 28-week period.

7) The distribution of parasite numbers/host during a 12-24 week infection period was overdispersed in both inbred and outbred mice.

8) The instantaneous rate of parasite mortality was found to differ between mouse strains, and was estimated at (/parasite/week): 0.10 in outbred MF1 mice; 0.11 in inbred NIH mice and 0.028 in inbred CBA/Ca mice. Thus, the average parasite life-span was about 9.9, 8.9 and 35.8 weeks in MF1, NIH and CBA/Ca mice, respectively. Mortality of the parasite was independent of age and only related to the dose of infection at very high parasite burdens.

9) The pattern of per capita egg production rate was found to be age-dependent within the experimental period of 12-24 weeks. Peak fecundity of H.polygyrus was 1240 eggs/female/16h and the estimated life-time

egg production by a female worm was approximately 18950 eggs in CBA/Ca mice.

10) Density-dependent per capita fecundity in H.polygyrus within the experimental range of infection doses used, was observed in both inbred and outbred mice.

11) Parasite establishment was reduced to 30% in 'immune' CBA/Ca mice compared to 55% in naive control mice. The instantaneous rate of parasite mortality in these immune mice was found to be 0.00075 ± 0.033, and was therefore comparable to the mortality rate in naive mice. The distribution of H.polygyrus/mouse in the immune CBA/Ca mice was highly overdispersed.

12) Mice exposed to large primary infections were protected against a large challenge infection administered after the removal of the primary infection by drug treatment.

13) The dynamics of repeated infection was different in high and low responder inbred mice during a 12-week exposure period. Low responder CBA/Ca mice accumulated worms in a dose-dependent manner. At low and medium infection intensities of 10 and 25 L3/mouse/week, respectively, there was an apparent loss of worms from 6 to 12 weeks after the initial infection, whereas mice exposed to the high dose of 50 L3/mouse/week continued to accumulate worms during the same period. An immigration-death model was used to describe the pattern of parasite intensity in these mice.

14) In high responder NIH mice, a convex age-intensity curve was generated at the high infection dose (50L3/mouse/week). A mathematical model incorporating the effects of acquired immunity on both parasite establishment and mortality was used to analyse the data.

15) The distribution of parasites/host in both the inbred and outbred mice during repeated infection became more overdispersed as the duration of exposure increased.

16) The proportion of mice infected during repeated exposure to infection was 100% in low responder mice, but declined to about 10% by the 12th week of infection in high responder mice.

17) More female than male worms were recovered during the latter period of the trickle infections in both strains of mice.

18) The per capita fecundity of H.polygyrus was stable at 600 eggs/female/16h in low responder mice, but was severely reduced (with a peak of 150 eggs/female,16h) in high responder mice during repeated infection.

19) The dynamics of repeated infection in CBA/Ca mice exposed to 50L3/mouse/2 weeks differed quantitatively from those in mice given weekly infections.

20) Outbred male MF1 mice exposed to repeated infection

exhibited dynamics of infection similar to a 'high' responder mouse strain.

21) Parasite establishment in inbred and outbred mice exposed to repeated infection was reduced in a manner related to the summed experience of past infection.

22) High responder mice were highly refractory to further infection following chemotherapy and vaccination with irradiated larvae.

23) Low responder mice were more resistant to further repeated infection when exposed to the high dose of trickle infection for 6 as opposed to 3 weeks of infection before drug treatment. The per capita fecundity of H.polygyrus in low responder mice was reduced significantly in mice given a 6-week initial trickle infection when compared with mice given a 3-week initial trickle infection.

24) Vaccination of low responders with irradiated larvae induced a high degree of resistance (60%) to repeated infection in these mice.

25) The resistance induced in low responder mice by both a repeated infection followed by chemotherapy and vaccination persisted at a high level of >95% for 7 weeks.

26) Serum anti-adult H.polygyrus antibodies of the IgG1 and IgM isotypes were significantly elevated when compared to naive mouse serum during repeated infection in both high and low responder hosts.

27) In low responders, anti-adult worm antibodies were higher in mice exposed to the high dose than the low doses of infection. At the high dose of infection, serum IgG1 antibodies to adult H.polygyrus were higher in low responders than in high responders from the second week to the end of the trickle infection period.

28) There was no apparent difference in the ability of sera from low and high responders exposed to repeated infection of H.polygyrus to 'recognize' the surface antigens of the fourth-stage larvae as assessed by immunoprecipitation of iodine-labelled surface antigens, SDS-PAGE and autoradiography.

29) The results were discussed in relation to the population dynamics and epidemiology of intestinal helminth infections in host communities.

REFERENCES

Ada, G.L. (1982). Vaccines for the future? Australian Journal of Experimental Biology and Medical Science 60, 549-569.

Adorini, L. & Doria, G. (1981). Defective antigen presentation by macrophages from mice genetically selected for low antibody response. European Journal of Immunology 11, 984-989.

Albers, G.A.A. & Gray, G.D. (1986). Breeding for worm resistance: a perspective. In: Parasitology - quo vadit? (ed.) M.J. Howell, pp. 559-566. Canberra.

Alghali, S.T.O., Hagan, P. & Robinson, M. (1985). Hymenolepis citelli (Cestoda) and Nematospiroides dubius (Nematoda): interspecific interaction in mice. Experimental Parasitology 60, 364-370.

Ali, N.M.H. & Behnke, J.M. (1984). Non-specific immuno-depression by Nematospiroides dubius of concurrent responses to oxazolone and lipopolysaccharide. Journal of Helminthology 58, 301-311.

Ali, N.M.H., Behnke, J.M. & Manger, B.R. (1985). The pattern of peripheral blood leucocyte changes in mice infected with Nematospiroides dubius. Journal of Helminthology 59, 83-93.

Alizadeh, H. & Murrell, K.D. (1984). The intestinal mast cell response to Trichinella spiralis infection in mast cell-deficient W/w^{v} mice. Journal of Parasitology 70, 767-773.

Alizadeh, H. & Wakelin, D. (1982). Genetic factors controlling the intestinal mast cell response in mice infected with Trichinella spiralis. Clinical and Experimental Immunology 49, 331-337.

Anderson, R.M. (1976). Dynamic aspects of parasite population ecology. In: Ecological Aspects of Ecology.(ed.) C.R. Kennedy, North-Holland Publishing Co., Amsterdam.

Anderson, R.M. (1978). The regulation of host population growth by parasitic species. Parasitology 76, 119-158.

Anderson, R.M. (1979). The influence of parasitic infection on the dynamics of host population growth. In: Population Dynamics.(eds.) R.M. Anderson, B.D. Turner & L.R. Taylor, pp.245-281. Blackwell Scientific Publications, Oxford.

Anderson, R.M. (1980). The dynamics and control of direct life cycle helminth parasites. Lecture Notes in Biomathematics 39, 278-322.

Anderson, R.M. (1981). Population ecology of infectious disease agents. In: Theoretical Ecology.(ed.) R.M. May, pp. 318-355, Blackwell Scientific Publications, Oxford.

Anderson, R.M. (1982a). Host-parasite population biology. In: Parasites - Their World and Ours.(eds.) D.F. Mettrick & S.S. Desser, pp. 303-312. Elsevier Biomedical Press, Amsterdam.

Anderson, R.M. (1982b). Population Dynamics of Infectious Diseases. Chapman & Hall, London.

Anderson, R.M. (1985). Mathematical models for the study of the epidemiology and control of ascariasis in man. In: Ascariasis and its Public Health Significance.(eds.) D.W.T. Crompton, M.C. Nesheim and Z.S. Pawlowski.pp. 39-68, Taylor and Francis, London.

Anderson, R.M. (1986). The role of mathematical models in helminth population biology. In: Parasitology-quo vadit? (ed.) M.J. Howell, pp. 519-529. Canberra.

Anderson, R.M. & Crombie, J.A. (1985). Experimental studies of age-intensity and age-prevalence profiles of infection: *Schistosoma mansoni* in snails and mice. In: *Ecology and Genetics of Host-Parasite Interactions*. (eds.) D. Rollinson & R.M. Anderson, pp.111-146. Academic Press, London.

Anderson, R.M. & Gordon, D.M. (1982). Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology 85*, 373-398.

Anderson, R.M. & May, R.M. (1978). Regulation and stability of host-parasite population interactions.I. Regulatory processes. *Journal of Animal Ecology 47*, 219-248.

Anderson, R.M. & May, R.M. (1982). Population dynamics of human helminth infections: control by chemotherapy. *Nature 297*, 557-563.

Anderson, R.M. & May, R.M. (1985a). Helminth infections of humans: mathematical models, population dynamics and control. *Advances in Parasitology 24*, 1-101.

Anderson, R.M. & May, R.M. (1985b). Herd immunity to helminth infection and implications for parasite control. *Nature 315*, 493-496.

Anderson, R.M. & May, R.M. (1985c). Vaccination and herd immunity to infectious diseases. *Nature 318*, 323-329.

Anderson, R.M. & Medley, G.F. (1985). Community control of helminth infections of man by mass and selective chemotherapy. *Parasitology 90*, 629-660.

Anderson, R.M. & Schad, G.A. (1985). Hookworm burdens and faecal egg counts: an analysis of the biological basis of variation. *Transactions of the Royal Society of Tropical Medicine and Hygiene 79*, 812-825.

Anderson, R.M. & Whitfield, P.J. (1975). Survival characteristics of the free-living cercarial population of the ectoparasitic digenean, Transversotrema patialense (Soparker,1924). Parasitology 70, 295-310.

Anderson, R.M., Whitfield, P.J., Dobson, A.P. & Keymer, A.E. (1978). Concomitant predation and infection processes: an experimental study. Journal of Animal Ecology 47, 891-911.

Areekul, S.(1975). Hookworm infection and anemia. Journal of the Medical Association of Thailand 58, 343-346.

Arnason, A.N., Dick, T.A. & Wassom, D.L. (1986). A model to assess survival mechanisms of parasites in a genetically defined host system. Parasitology 92, 253-267.

Asch, H.L. & Drane, W. (1972). Schistosoma mansoni: analysis of the kinetics of death of parasitic organisms by employing attrition of cercariae as a model. Experimental Parasitology 31, 284-289.

Bailey, D.W. (1982). How pure are inbred strains of mice? Immunology Today 3, 210-214.

Baird, J.K., Mistrey, M., Pimsler, M. & Connor, D.H. (1986). Fatal human ascariasis following secondary massive infection. American Journal of Tropical Medicine and Hygiene 35, 314-318.

Barger, I.A. (1986). Population regulation in trichostrongylids of ruminants. In: Parasitology-Quo Vadit? (ed.) M.J. Howell, pp. 531-539. Canberra.

Barger, I.A., LeJambre, L.F., Georgi, J.R. & Davies, H.I. (1985). Regulation of Haemonchus contortus populations in sheep exposed to continuous infection. International Journal for Parasitology 15, 529-533.

Barriga, O.O. (1981). The Immunology of Parasitic Infections. M.T.P. Press, Lancaster.

Bartlett, A. & Ball, P.A.J. (1972). Nematospiroides dubius in the mouse as a possible model of endemic human hookworm infection. Annals of Tropical Medicine and Parasitology 66, 129-134.

Bartlett, A. & Ball, P.A.J. (1974). The immune response of the mouse to larvae and adults of Nematospiroides dubius. International Journal for Parasitology 4,463-470.

Bawden, R.J. (1969). Some effects of the diet of mice on Nematospiroides dubius (Nematoda). Parasitology 59, 203-213.

Baylis, H.A. (1926). On a trichostrongylid nematode from the wood mouse (Apodemus sylvaticus). Ann. Mag. nat. Hist. 18, 455-464.

Baylis, H.A. (1927). A further note on Nematospiroides dubius Baylis 1926. Ann. Mag. nat. Hist. 20, 102-105.

Befus, A.D & Bienenstock, J. (1979). Immunologically mediated intestinal mastocytosis in Nippostrongylus brasiliensis-infected rats. Immunology 38, 95-101.

Befus, A.D. & Bienenstock, J. (1984). Induction and expression of mucosal immune responses and inflammation to parasitic infections. In: Immunobiology of Parasites and Parasitic Infections. Vol. 12. (ed.) J.J. Marchalonis, Plenum Press, New York.

Behnke, J.M., Ali, N.M.H. & Jenkins, S.N. (1984). Survival to patency of low-level infections with Trichuris muris in mice concurrently infected with Nematospiroides dubius. Annals of Tropical Medicine and Hygiene 78, 509-517.

Behnke, J.M. & Hannah, J. (1984). Nematospiroides dubius in the jird, Meriones unguiculatus: stimulation and expression of acquired immunity. Journal of Helminthology 58, 221-229.

Behnke, J.M., Hannah, J. & Pritchard, D.I. (1983). Nematospiroides dubius in the mouse: evidence that adult worms depress the expression of homologous immunity. Parasite Immunology 5, 397-408.

Behnke, J.M. & Parish, H.A. (1980). The effect of gamma irradiation on Nematospiroides dubius. Factors affecting the survival of worms in a primary infection in mice. Journal of Helminthology 54, 173-182.

Behnke, J.M. & Parish, H.A. (1979a). Nematospiroides dubius: arrested development of larvae in immune mice. Experimental Parasitology 47, 116-127.

Behnke, J.M. & Parish, H.A. (1979b). Expulsion of Nematospiroides dubius from the intestine of mice treated with immune serum. Parasite Immunology 1, 13-26.

Behnke, J.M. & Parish, H.A. (1981). Transfer of immunity to Nematospiroides dubius: co-operation between lymphoid cells and antibody in mediating worm expulsion. Parasite Immunology 3, 249-259.

Behnke, J.M., Paul, V. & Rajasekariah, G.R. (1986). The growth and migration of Necator americanus following infection in neonatal hamsters. Transactions of the Royal Society of Tropical Medicine and Hygiene 80, 146-149.

Behnke, J.M. & Robinson, M. (1985). Genetic control of immunity to Nematospiroides dubius: a 9-day anthelmintic abbreviated immunizing regime which separates weak and strong responder strains of mice. Parasite Immunology 7, 235-253.

Behnke, J.M. & Wakelin, D. (1973). The survival of Trichuris muris in wild populations of its natural hosts. Parasitology 67, 157-164.

Behnke, J.M. & Wakelin, D. (1977). Nematospiroides dubius: stimulation of acquired immunity in inbred strains of mice. Journal of Helminthology 51, 167-175.

Behnke, J.M., Wakelin, D. & Wilson, M.M. (1978). Trichinella spiralis: delayed rejection in mice concurrently infected with Nematospiroides dubius. Experimental Parasitology 46, 121-130.

Berding, C., Murray, J.D., Slater, A.F.G. & Keymer, A.E. (1986). The population dynamics of acquired immunity to helminth infection. Journal of Theoretical Biology (in press).

Bensted-Smith, R., Anderson, R.M., Butterworth, A.E., Capron, M., Cordingley, J.S., Dalton, P.R., Dunne, D.W., Karinki, H.C., Kimani, G., Koech, D., Mugambi, M., Ouma, J.H., Prentice, M.A., Richardson, B.A., Siongok, T.K., Sturrock, R.F. & Taylor, D.W. (1986). Predisposition to Schistosoma mansoni infection in children. Transactions of the Royal Society of Tropical Medicine and Hygiene. (in press).

Biozzi, G., Mouton, D., Sant'Anna, O.A., Passos, H.C., Gennari, M., Reis, M.H., Ferreira, V.C.A., Heumann, A.M., Bouthillier, Y., Ibanez, O.M., Stiffel, C. & Sequeira, M. (1979). Genetics of immuneresponsiveness to natural antigens in the mouse. Current Topics in Microbiology and Immunobiology 85, 33-98.

Blackwell, J.M. (1985). A murine model of genetically controlled host responses to leishmaniasis. In: Ecology and Genetics of Host-Parasite Interactions. (eds.) D. Rollinson & R.M. Anderson, pp. 147-156, Academic Press, London.

Bliss, C.A. & Fisher, R.A. (1953). Fitting the negative binomial distribution to biological data and a note on the efficient fitting of the negative binomial. Biometrics 9, 176-200.

Boulenger, C.L. (1922). The structure and systematic position of Strongylus polygyrus. Parasitology 14,206-13.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248-254.

Bradley, D.J. (1972). Regulation of parasite populations. A general theory of the epidemiology and control of parasitic infections. Transactions of the Royal Society of Tropical Medicine and Hygiene 66, 697-708.

Bradley, D.J. (1974). Stability in host-parasite systems. In: Ecological Stability, (eds.) M.B. Usher & M.H. Williamson. Chapman and Hall, London.

Bradley, D.J. & May, R.M. (1978). Consequences of helminth aggregation for the dynamics of schistosomiasis. Transactions of the Royal Society of Tropical Medicine Hygiene 72, 262-273.

Brindley, P.J. & Dobson, C. (1981). Genetic control of liability to infection with Nematospiroides dubius in mice: selection of refractory and liable populations of mice. Parasitology 83, 51-65.

Brindley, P.J. & Dobson, C. (1982). Multiple infections with Nematospiroides dubius in mice selected for liability to a single infection. Australian Journal of Experimental Biology and Medical Science 60, 319-327.

Brindley, P.J. & Dobson, C. (1983). Genetic control of liability to infection with Nematospiroides dubius in mice: direct and correlated responses to selection of mice for faecal parasite egg count. Parasitology 87, 113-127.

Brindley, P.J. & Dobson, C. (1983). Partitioning innate and acquired immunity in mice after infection with Nematospiroides dubius. International Journal for Parasitology 13, 503-507.

Brindley, P.J., He, S., Sitepu, P., Pattie, W.A. & Dobson, C. (1986). Inheritance of immunity in mice to challenge infection with Nematospiroides dubius. Heredity 57, 53-58.

Brindley, P.J., Prociv, P., Creevey, C.A. & East, I.J. (1985). Regulation of toxocariasis in mice selectively reared for high and low immune responses to Nematospiroides dubius. Journal of Helminthology 59, 157-166.

Brown, A.R., Crandall, R.B. & Crandall, C.A. (1976). Increased IgG catabolism as a possible factor in the immunosuppression produced in mice infected with Heligmosomoides polygyrus. Journal of Parasitology 62, 169-171.

Bryant, V. (1973). The life cycle of Nematospiroides dubius, Baylis,1926 (Nematoda: Heligmosomidae). Journal of Helminthology 47, 263-268.

Bullick, G.R., Russell, D.A. & Castro, G.A. (1984). Long term duration of the rapid rejection response in rats infected with Trichinella spiralis. Journal of Parasitology 70, 981-982.

Bulmer, M.G. (1980). The Mathematical Theory of Quantitative Genetics. Oxford University Press, Oxford.

Bundy, D.A.P. (1985). Parasitic worms in the aetiology of iron deficiency anaemia in the Caribbean region. The Caribbean Food and Nutrition Institute Quarterly 18, 197-215.

Bundy, D.A.P., Cooper, E.S., Thompson, D.E., Didier, J.M., Anderson, R.M. & Simmons, I. (1985). Predisposition to Trichuris trichiura infection in humans. Journal of Hygiene (in press).

Butler, R.G. (1980). Population size , social behaviour and dispersal in house mice: a quantitative investigation. Animal Behaviour 28, 78-85.

Butterworth, A.E. (1980). Eosinophils and immunity to parasites. Royal Society of Tropical Medicine and Hygiene 74 (suppl.), 38

Carr, A. & Pritchard, D.I. (1986). Identification of hookworm (Necator americanus) antigens and their translation in vitro. Molecular and Biochemical Parasitology 19, 251-258.

Carroll, S.M. & Grove, D.I. (1984). Parasitological, hematologic and immunologic responses in acute and chronic infections of dogs with Ancylostoma ceylanicum: a model of human hookworm infection. Journal of Infectious Diseases 150, 284-294.

Carroll, S.M. & Grove, D.I. (1985). Resistance of dogs to reinfection with Ancylostoma ceylanicum following anthelmintic therapy. Transactions of the Royal Society of Tropical Medicine and Hygiene 79, 519-523.

Cayzer, C.J.R. & Dobson, C. (1983). Suppression of antibody production in mice given multiple concurrent infections with Nematospiroides dubius. International Journal for Parasitology 13, 61-65.

Chaicumpa, V., Jenkin, C.R. & Fischer, H. (1977). The effect *in vivo* of peritoneal exudate cells of immune and normal mice on the infectivity of the third stage larvae of *Nematospiroides dubius*. Australian Journal of Experimental Biology and Medical Science 55, 561.

Chaicumpa, V. & Jenkin, C.R. (1978). Studies *in vitro* on reaction of peritoneal exudate cell from mice immune to infection with *Nematospiroides dubius* with the infective third stage larvae of the parasite. Australian Journal of Experimental Biology and Medical Science 56, 61-68.

Chaicumpa, V., Jenkin, C.R. & Rowley, D. (1976). Passive transfer of immunity to infection with *Nematospiroides dubius* from immunized mice to their offspring. Australian Journal of Experimental Biology and Medical Science 54, 245.

Chaicumpa, V., Prowse, S.J., Ey P.L. & Jenkin, C.R. (1977). Induction of immunity in mice to the nematode parasite, *Nematospiroides dubius*. Australian Journal of Experimental Biology and Medical Science 55, 393-400.

Chapman, C.B., Knopf, P.M., Anders, R.F. & Mitchell, G.F. (1979a). IgG1 hypergammaglobulinaemia in chronic parasite infections in mice. Evidence that the response reflects chronicity of antigen exposure. Australian Journal of Experimental Biology and Medical Science 57, 389.

Chapman, C.B., Knopf, P.M., Hicks, J.D. & Mitchell, G.F. (1979b). IgG1 hypergammaglobulinaemia in chronic parasite infections in mice. Magnitude of the response in mice infected with various parasites. Australian Journal of Experimental Biology and Medical Science 57, 369.

Chowaniec, W., Wescott, R.B. & Congdon, L.L. (1972). Interaction of Nematospiroides dubius and influenza virus in mice. Experimental Parasitology 32, 33-44.

Chowdhury, A.B. & Schiller, E.L. (1968). A survey of parasitic infections in a rural community near Calcutta. American Journal of Epidemiology 87, 299-312.

Clegg, J.A. & Smith, M.A. (1978). Prospects for the development of dead vaccines against helminths. Advances in Parasitology 16, 165-218.

Cohen, S. & Warren, K.S. (eds.)(1982). Immunity to Parasitic Infections. Blackwell Scientific Publication, Oxford.

Connan, R.M. (1970). The effect of host lactation on the 'self cure' of Nippostrongylus brasiliensis in rats. Parasitology 61, 27-33.

Connan, R.M. (1972). The effect of host lactation on the second infection of Nippostrongylus brasiliensis in rats. Parasitology 64, 229-233.

Conway, G.R., Wilcox, J.C. & Glass, N.R. (1970). Fitting non-linear models to biological data by Marquardts' algorithm. Ecology 51, 503-508.

Cort, W.W., Schapiro, L. & Stoll, N.R. (1929). A study of re-infection after treatment with hookworm and ascaris in two villages in Panama. American Journal of Hygiene 10, 614-625.

Cox, F.E.G. (1978). Specific and non-specific immunisation against parasitic infections. Nature 273, 623-626.

Crandall, R.B., Crandall, C.A. & Franco, J.A. (1974). Heligmosomoides polygyrus: humoral and intestinal immunologic responses to infection in mice. Experimental Parasitology 35, 275-287.

Crofton, H.D. (1971). A quantitative approach to parasitism. Parasitology 62, 179-194.

Croll, N.A., Anderson, R.M., Gyorkos, T.W. & Ghadirian, E. (1982). The population biology and control of Ascaris lumbricoides in a rural community in Iran. Transactions of the Royal Society of Tropical Medicine and Hygiene 76, 187-197.

Croll, N.A. & Ghadirian, E. (1981). Wormy persons: contributions to the nature and patterns of overdispersion with Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus and Trichuris trichiura. Tropical and Geographical Medicine 33, 241-248.

Cross, J.H. & Duffy, C.E. (1963). Nematospiroides dubius in the abnormal host. Annals of the New York Academy of Science 113, 88-99.

Cross, J.H. & Scott, J.A. (1960). Nematospiroides dubius in in the Mongolian gerbil. Journal of Parasitology 46, 38.

Crowle, P.K. & Reed, N.D. (1981). Rejection of the intestinal parasite Nippostrongylus brasiliensis by mast cell-deficient w/w^{v} mice. Infection and Immunity 33, 54-58.

Cypess, R. (1970). Artificial production of acquired immunity in mice by footpad injections of crude larval extract of Nematospiroides dubius. Journal of Parasitology 56, 320.

Cypess, R. (1972). Blood cell changes associated with immunity to Nematospiroides dubius. Journal of Parasitology 58, 563-566.

Cypess, R.H., Ebersole, J.L. & Molinari, J.A. (1977). Specific antibody levels in the intestinal perfusates of Heligmosomoides polygyrus-infected mice. International Archives of Allergy and Applied Immunology 55, 496-503.

Cypess, R.H., Van Zandt, P., & Zidian, J.L. (1973). Development of immunity in mice previously infected at an early age with Nematospiroides dubius. Journal of Parasitology 59, 776-779.

Cypess, R.H. & Zidian, J.L. (1975). Heligmosomoides polygyrus: the development of self-cure and/or protections in several strains of mice. Journal of Parasitology 61, 819-824.

Dargie, J.D. (1982). The influence of genetic factors on the resistance of ruminants to gastrointestinal nematode and trypanosome infections. In: Animal Models in Parasitology, (ed) D.G. Owen, pp. 17-51. McMillan, London.

Dean, D.A. (1983). A review. Schistosoma and related genera: acquired resistance in mice. Experimental Parasitology 55, 1-104.

De Fries, J.C. & McClearn, G.E. (1970). Social dominance and darwinian fitness in the laboratory mouse. American Naturalist 104, 408-411.

Denham, D.A., Ponnudurai, T., Nelson, G.S., Guy, F. & Rogers, R. (1972). Studies with Brugia pahangi.I. Parasitological observations on primary infections of cats (Felis catus). International Journal for Parasitology 2, 239-247.

Dineen, J.K. & Kelly, J.D. (1972). The suppression of rejection of Nippostrongylus brasiliensis in lactating rats: the nature of the immunological defect. Immunology 22, 1-12.

Dineen, J.K. & Windon, R.G. (1980). The effect of sire selection on the response of lambs to vaccination with irradiated Trichostrongylus colubriformis larvae. International Journal for Parasitology 10, 189-196.

Dobson, C. (1961). Certain aspects of the host-parasite relationship of Nematospiroides dubius (Baylis). II. The effect of sex on experimental infections in the rat. Parasitology 51, 499-510.

Dobson, C. (1982). Passive transfer of immunity with serum in mice infected with Nematospiroides dubius: influence of quality and quantity of immune serum. International Journal for Parasitology 12, 207-213.

Dobson, C. & Cayzer, C.J.R. (1982). Passive transfer of immunity with serum in mice infected with Nematospiroides dubius: in vitro effect of immune serum on larval infectivity. International Journal for Parasitology 12, 413-421.

Dobson, C. & Owen, M.E. (1978). Effect of host sex on passive immunity in mice infected with Nematospiroides dubius. International Journal for Parasitology 8, 359-364.

Dobson, C., Brindley, P.J. & Sitepu, P. (1982). Influence of serum donor and recipient mouse genotype on the passive transfer of protective immunity with serum against Nematospiroides dubius. International Journal for Parasitology 12, 567-572.

Dobson, C., Sitepu, P. & Brindley, P.J. (1985). Influence of primary infection on the population dynamics of Nematospiroides dubius after challenge infections in mice. International Journal for Parasitology 15, 353-359.

Docherty, J.F. (1926). Hookworm infestation and re-infestation in Ceylon. A study of high incidence with a moderate degree of infestation in Ceylon. American Journal of Hygiene 6, 160-171.

Dujardin, F. (1845). Histoire Naturelle des Helminthes ou Vers Intestinaux. Paris.

Durette-Desset, M.-C. (1985). Trichostrongylid nematodes and their vertebrate hosts: reconstruction of the phylogeny of a parasitic group. Advances in Parasitology 24, 239-306.

Durette-Desset, M.-C., Kinsella, J.M. & Forrester, D.J. (1972). Arguments en faveur de la double origine des nematodes nearctiques du genre Heligmosomoides Hall,1916. Annales de Parasitologie 47, 365-382.

Ehrenford, F.A. (1954). The life cycle of Nematospiroides dubius, Baylis (Nematoda: Heligmosomidae). Journal of Parasitology 40, 480-481.

Elkins, D., Haswell-Elkins, M. & Anderson, R.M. (1986). The epidemiology and control of intestinal helminths in the Pulicat Lake region of Southern India. I. Study design and pre- and post-treatment observations on Ascaris lumbricoides infection. Transactions of the Royal Society of Tropical Medicine and Hygiene (in press).

Elliott, J.M. (1977). Some Methods for the Statistical Analysis of Samples of Benthic Invertebrates. Freshwater Biological Association, 5, Titus Wilson & Sons., Kendal.

Elton, C., Ford, E.B. & Baker, J.R. (1931). The health and parasites of a wild mouse population. Proceedings of the Zoological Society of London 1931, 657-721.

Engvall, E. & Perlmann, P. (1972). ELISA. III. Quantitation of specific antibodies by enzyme linked anti-immunoglobulin in antigen coated tubes. Journal of Immunology 109, 129-135.

Ey, P.L., Prowse, S.J. & Jenkin, C.R. (1981). Heligmosomoides polygyrus: simple recovery of post-infective larvae from mouse intestines. Experimental Parasitology 52,69-76.

Fahmy, M.A.M. (1956). An investigation on the life cycle of Nematospiroides dubius (Nematoda: Heligmosomidae) with special reference to the free-living stages. Zeitschrift fur Parasitenkunde 17, 394-399.

Falconer, D.S. (1981). Introduction to Quantitative Genetics. Longman, London.

Fernando, M.A. & Balasuriya, S. (1976). Prevalence of woil-transmitted helminths in the Hindagala community health project area. Ceylon Medical Journal 22,177-183.

Fernando Monroy, G., Brindley, P.J. & Dobson, C. (1985). Vaccination against Nematospiroides dubius in mice using adult worm extracts as antigens. Australian Journal of Experimental Biology and Medical Science 63, 423-430.

Festing, M.F.W. (1982). Genetic manipulation of the host as a method in parasitology. In: Animal Models in Parasitology, (ed.) D.G. Owen, pp. 1-16. MacMillan,London.

Flowerdew, J.R. (1985). The population dynamics of wood mice and yellow-necked mice. In: Ecology of Woodland Rodents, Bank Voles and Wood Mice. (eds.) J.R. Flowerdew, J. Gurnell & J.H.W. Gripps, pp. 315-338. Clarendon Press, Oxford.

Forrester, D.J. & McL. Neilson, J.T. (1973). Comparative infectivity of Heligmosomoides polygyrus in three species of Peromyscus. Journal of Parasitology 59,251-255.

Fraker, P.J. & Speck, J.C. (1978). Protein and cell membrane iodination with a sparingly soluble chloro-amide 1,3,4,6-tetrachloro-3α-6α diphenylglycouril. Biochemical and Biophysical Research Communications 80, 849.

Freeland, W.J. (1981). Parasitism and behavioural dominance among male mice. Science 213, 461-462.

Garg, M.L., Raja, R.B. & Redmond, C.K. (1970). Maximum likelihood estimation of the parameters of the Gompetz survival function. Applied Statistics 19, 152-159.

Georgi, J.R. & Le Jambre, L.F. (1983). Radiolabelling of infective larvae of Haemonchus contortus (Nematoda: Trichostrongyloidea) with ^{75}Se-methionine and their performance as tracers in sheep. Journal of Parasitology 69, 875-882.

Gibson, G.W., Jacobson, R.H. & Sayles, P.C. (1983). Nematospiroides dubius: characterization of infections in mice using radiotracer techniques. Proceedings of the American Society of Parasitology 38.

Gilles, H.M., Watson-Williams, E.J. & Ball, P.A.J. (1964). Hookworm infection and anaemia: an epidemiological, clinical and laboratory study. Quarterly Journal of Medicine 33, 1-24.

Gleich, G.J. & Loegering, D.A. (1984). Immunobiology of eosinophils. Annual Review of Immunology 2, 429.

Goven, A.J. & De Buysscher, V. (1980). Production of immunity in mice to Nematospiroides dubius by use of a double-emulsion adjuvant. Journal of Parasitology 66, 346-347.

Green, E.L. (1981). Genetics and Probability in Animal Breeding Experiments. McMillan Press, London.

Grencis, R.K., Crawford, C., Pritchard, D.I., Behnke, J.M. & Wakelin, D. (1986). Immunization of mice with surface antigens from the infective L1 muscle larvae of Trichinella spiralis. Parasite Immunology (in press).

Ha, Y.T., Reed, N.D. & Crowle, P.K. (1983). Delayed expulsion of adult Trichinella spiralis by mast cell deficient w/w^{v} mice. Infection and Immunity 41,445-447.

Hagan, P., Behnke, J.M. & Parish, H.A. (1981). Stimulation of immunity to Nematosipiroides dubius in mice using larvae attenuated by cobalt 60 irradiation. Parasite Immunology 3, 149-156.

Hagan, P. & Wakelin, D. (1982). Nematospiroides dubius: effect of infection on lymphocyte responses to Trichinella spiralis in mice. Experimental Parasitology 54, 157-165.

Haley, A.J. (1962). Role of host relationships in the systematics of helminth parasites. Journal of Parasitology 48, 671-678.

Hall, A. (1981). Quantitative variability of nematode egg counts in faeces: a study among rural Kenyans. Transactions of the Royal Society of Tropical Medicine and Hygiene 75, 682-687.

Hall, A. (1982). Intestinal helminths of man: the interpretation of egg counts. Parasitology 85, 605-613.

Hall, M.C. (1916). Nematode parasites of mammals of the Orders Rodentia, Lagomorpha and Hyracoidea. Proceedings of the United States National Museum 1, 1-258.

Hannah, J. & Behnke, J.M. (1982). Nematospiroides dubius in the jird, Meriones unguiculatus: factors affecting the course of a primary infection. Journal of Helminthology 56, 329-338.

Haswell-Elkins, Elkins, D. & Anderson, R.M. (1986). The epidemiology and control of intestinal helminths in the Pulicat Lake region of Southern India. Predisposition to heavy or light infection. Journal of Infectious Diseases (in press).

Healing, T.D. & Nowell, F. (1985). Diseases and parasites of woodland rodent populations. In: The Ecology of Woodland Rodents, Bank Voles and Wood Mice, (eds.) J.R. Flowerdew, J. Gurnell & J.H.W. Gripps, pp. 193-208, Clarendon Press, Oxford.

Hepler, D.I., Lueker, D.C. & Rubin, R. (1976). The efficacy oral immunization with Nematospiroides dubius/mouse model. Journal of Parasitology 62, 491-492.

Hill, B.R. (1926). Hookworm re-infestation for three years after treatment in a sanitated area in Puerto Rico and its bearing on permanent hookworm control in the group studied. American Journal of Hygiene 6, 103-117.

Hill, W.G. (1971). Design and efficiency of selection experiments for estimating genetic parameters. Biometrics 27, 293-311.

Hill, W.G. (1972a). Estimation of realized heritabilities from selection experiments. I. Divergent selection. Biometrics 28, 747-765.

Hill, W.G. (192b). Estimation of realized heritabilities from selection experiments. II. Selection in one direction. Biometrics 28, 767-780.

Hoagland, K.E. & Schad, G.A. (1978). Necator americanus and Ancylostoma duodenale: life history parameters and epidemiological implications of two sympatric hookworms of man. Experimental Parasitology 44, 36-49.

Hohenboken, W.D. (1985). Prediction and measurement of response to selection. In: General and Quantitative Genetics, (ed.) A.B. Chapman, pp. 167-186. Elsevier, Amsterdam.

Holmes, J.C. (1982). Impact of infectious disease agents on the population growth and geographical distribution of animals. In: Population Biology of Infectious Disease Agents, (eds.) R.M. Anderson & R.M. May, Springer, Berlin.

Holmes, J.C. & Bethel, W.W. (1972). Modification of intermediate host behaviour by parasites. In: Behavioural Aspects of Parasite Transmission, (eds.) E.U. Canning & C.A. Wright. Academic Press, London.

Hominick, W., Dean, C.G. & Schad, G.A. (1986).(in preparation).

Hood, L.E., Weissman, I.L., Wood, W.B. & Wilson, J.H. (1984). Immunology. Benjamin Cummings, California.

Hosier, D.W. (1974). Immunity of male ICR mice to Nematospiroides dubius. Journal of Parasitology 60, 735.

Hosier, D.W. & Feller, M.D. (1973). Acquired immunity to Nematospiroides dubius in ICR mice. Journal of Parasitology 59, 751-752.

Hosier, D.W., Sackman, I., Idell, S. (1974). Vaccination of ICR mice with various doses of Nematospiroides dubius larvae. Journal of Parasitology 60, 555-556.

Hotez, P.J. & Cerami, A. (1983). Secretion of a proteolytic anticoagulant by Ancylostoma hookworms. Journal of Experimental Medicine 157, 1594-1603.

Hotez, P.J., LeTrang, N., McKerrow, J.H. & Cerami, A. (1985). Isolation and characterization of a proteolytic enzyme from the adult hookworm Ancylostoma caninum. Journal of Biological Chemistry 260, 7343-7348.

Hsieh, H.C. (1970). Studies on endemic hookworm. 2. Comparison of the efficacy of anthelmintics in Taiwan and Liberia. Japanese Journal of Parasitology 19, 534-536.

Hurley, J.C., Day, K.P. & Mitchell, G.F. (1980). Accelerated rejection of Nematospiroides dubius intestinal worms in mice sensitized with adult worms. Australian Journal of Experimental Biology and Medical Science 58, 231-240.

Hurley, J.C. & Vadas, M.A. (1983). Eosinophilia and acquisition of resistance to Nematospiroides dubius in mice sensitized with adult worms. Australian Journal of Experimental Biology and Medical Science 61, 1-9.

Ibarra, O.F. & Jenkins, D.C. (1984). The relevance of in vitro anthelmintic screening tests employing the free-living stages of trichostrongylid nematodes. Journal of Helminthology 58, 107-112.

Jacobson, R.H., Brooks, B.O. & Cypess, R.H. (1982). Immunity to Nematospiroides dubius: parasite stages responsible for and subject to resistance in high responder (LAF_1/J) mice. Journal of Parasitology 68, 1053-1058.

Jarrett, E.E.E., Jarrett, W.F.H. & Urguhart, G.M. (1968). Qualitative studies on the kinetics of establishment and expulsion of intestinal nematode populations in susceptible and immune hosts. Nippostrongylus brasiliensis in the rat. Parasitology 58, 625-639.

Jarrett, E., Mackenzie, S. & Benwich, H. (1980). Parasite-induced 'non-specific' IgE does not protect against allergic reactions. Nature 283, 302-304.

Jenkins, D.C. (1975). The influence of Nematospiroides dubius on subsequent Nippostrongylus brasiliensis infections in mice. Parasitology 71, 349-355.

Jenkins, D.C. (1977). Nematospiroides dubius: the course of primary and challenge infections in the jird, Meriones unguiculatus. Experimental Parasitology 41, 335-340.

Jenkins, D.C. (1982). In vitro screening tests for anthelmintics. In: Animal Models in Parasitology, (ed.) D.G. Owen. McMillan Press, London.

Jenkins, S.N. & Behnke, J.M. (1977). Impairment of primary expulsion of Trichuris muris in mice concurrently infected with Nematospiroides dubius. Parasitology 75, 71-78.

Jenkins, D.C. & Phillipson, R.F. (1971). The kinetics of repeated low-level infections of Nippostrongylus brasiliensis in the laboratory rat. Parasitology 62, 457-465.

Jenkins, D.C. & Phillipson, R.F. (1972). Increased establishment and longevity of Nippostrongylus brasiliensis in immune rats given repeated small infections. International Journal for Parasitology 2, 105-111.

Jones, C.E. (1974). Nematospiroides dubius: mechanisms of host immunity. II. Skin testing and immunosuppression of orally or subcutaneously sensitized mice. Experimental Parasitology 35, 453-464.

Jones, C.E. & Rubin, R. (1974). Mechanisms of immunity to Nematospiroides dubius. I. Parasite counts, histopathology and serum transfer involving orally or subcutaneously sensitized mice. Experimental Parasitology 35, 434-452.

Jones, J.T. & Kusel, J.R. (1985). The inheritance of responses to schistosomiasis mansoni in two pairs of inbred strains of mice. Parasitology 90, 289-300.

Jungery, M. & Ogilvie, B.M. (1982). Antibody response to stage-specific Trichinella spiralis surface antigens in strong and weak responder mouse strains. Journal of Immunology 129, 839-843.

Kassai, T. (1982). Handbook of Nippostrongylus brasiliensis (Nematoda). Commonwealth Agricultural Bureaux.

Kay, A.B. (1985). Eosinophils as effector cells in immunity and hypersensitivity disorders. Clinical and Experimental Immunology 62, 1-12.

Kennedy, C.R. (1985). Interactions of fish and parasite populations: to perpetuate or pioneer? In: Ecology and Genetics of Host-Parasite Interactions. (eds.) D. Rollinson & R.M. Anderson, pp.1-20. Academic Press, London.

Kennedy, M.W., Qureshi, F., Haswell-Elkins, M. & Elkins, D. (1986). Homology and heterology between the secreted antigens of the parasitic larval stages of Ascaris lumbricoides and Ascaris suum. Clinical and Experimental Immunology (in press).

Kerboeuf, D.(1978a). The effects of time and temperature of storage on the infectivity of third-stage larvae of Heligmosomoides polygyrus. I. Effects on the development to the adult stage in mice. Annales des Recherches Veterinaire 9, 153-159.

Kerboeuf, D. (1978b). The effects of time and temperature of storage on the infectivity of third-stage larvae of Heligmosomoides polygyrus. II. Studies on the fecundity of female worms as a function of the infectivity of the third stage larvae. Annales des Recherches Veterinaire 9, 161-168.

Kerboeuf, D. (1982). Egg output of Heligmosomoides polygyrus in mice infected once only. Annales des Recherches Veterinaires 13, 69-78.

Kerboeuf, D. & Jacòb, C. (1983). Rhythmic egg release by Heligmosomoides polygyrus, parasitic Trichostrongylida of the murine gut. Chronobiologia 10, 255-267.

Kerboeuf, D. & Jolivet, G. (1980). Repeated anthelmintic treatments and receptivity of mice to experimental infections with Heligmosomoides polygyrus. Annales des Recherches Veterinaires 11, 185-193.

Kerboeuf, D. & Jolivet, G. (1984). Heligmosomoides polygyrus: time of anthelmintic treatment and infection parameters in mice exposed to increasing doses of larvae. Experimental Parasitology 57, 307-315.

Keymer, A.E. (1982). Density-dependent mechanisms in the regulation of intestinal helminth populations. Parasitology 84, 577-587.

Keymer, A.E. (1985). Experimental epidemiology: Nematospiroides dubius and the laboratory mouse. In: Ecology and Genetics of Host-Parasite Interactions. (eds.) D. Rollinson & R.M. Anderson, pp. 55-76. Academic Press, London.

Keymer, A.E. & Anderson, R.M. (1979). The dynamics of infection of Tribolium confusum by Hymenolepis diminuta: the influence of infective stage density and spatial distribution. Parasitology 79, 195-207.

Keymer, A.E. & Hiorns, R.W. (1986a). Faecal egg counts and nematode fecundity: Heligmosomoides polygyrus and laboratory mice. Parasitology 93, 189-204.

Keymer, A.E. & Hiorns, R.W. (1986b). Heligmosomoides polygyrus: the dynamics of primary and repeated infection in outbred mice. Proceedings of the Royal Society of London (in press).

Kojima, S., Kitamura, Y. & Takatsu, K. (1980). Prolonged infection of Nippostrongylus brasiliensis in genetically mast cell-deficient w/w^{v} mice. Immunology Letters 2, 159-162.

Kuns, M.L. & Rausch, R. (1950). An ecological study of helminths of some Wyoming voles (Microtus spp.) with a description of a new species of Nematospiroides (Heligmosomidae: Nematoda). Zoologica, New York Zoological Society 35, 181-188.

Langley, R. & Fairley, J.s. (1982). Seasonal variations in infestations of parasites in a wood mouse Apodemus sylvaticus population in the West of Ireland. Journal of Zoology, London,198, 249-261.

Lee, D.L. (1972). The structure of the helminth cuticle. Advances in Parasitology 10, 347-379.

Lee, D.L. & Aitkinson, H.J. (1976). Physiology of Nematodes. McMillan Press, London.

Lewis, J.W. (1968a). Studies on the helminth parasites of the long-tailed field mouse, Apodemus sylvaticus sylvaticus from Wales. Journal of Zoology (London) 154, 287-312.

Lewis, J.W. (1968b). Studies on the helminth parasites of voles and shrews from Wales. Journal of Zoology (London) 154, 313-331.

Lewis, J.W. & Bryant, V. (1976). The distribution of Nematospiroides dubius within the small intestine of laboratory mice. Journal of Helminthology 50, 163-171.

Lewis, J.W. & Shava, F.H.M. (1977). Rhythmic egg deposition by intestinal nematodes of laboratory mice. Parasitology 75, 2.

Lewis, J.W. & Twigg, C.I. (1972). A study of the internal parasites of small rodents from woodland areas in Surrey. Journal of Zoology (London) 166, 61-77.

Lidicker, W.Z. (1975). The role of dispersal in the demography of small mammals. In: Small Mammals: their Productivity and Population Dynamics. Cambridge University Press, Cambridge.

Liu, S.-K. (1966). Genetic influence on resistance of mice to Nematospiroides dubius. Experimental Parasitology 18, 311-319.

Liu, S.-K., Cypess, R.H. & Van Zandt, P. (1974). Gastritis caused by multiple Nematospiroides dubius infections. Journal of Parasitology 60, 790-795.

Lloyd, S. (1981). Progress in immunization against parasitic helminths. Parasitology 83, 225-242.

Lueker, D.C. & Hepler, D.I. (1975). Differences in immunity to Nematospiroides dubius in inbred and outbred mice. Journal of Parasitology 61, 158-159.

Lueker, D.C., Rubin, R. & Andersen, S. (1968). Protection of mice against Nematospiroides dubius by subcutaneously administered larval vaccines. Journal of Parasitology 54, 1237-1238.

Luzatto, L., Usanga, E.A. & Modiano, G. (1985). Genetic resistance to Plasmodium falciparum: studies in the field and in cultures in vitro. In: Ecology and Genetics of Host-Parasite Interactions, (eds.) D. Rollinson & R.M. Anderson, pp. 205-214. Academic Press, London.

MacDonald, G. (1965). The dynamics of helminth infections, with special reference to schistosomes. Transactions of the Royal Society of Tropical Medicine and Hygiene 59, 489-506.

Maizels, R., Burke, J., Sutanto, I. & Partono, P. & F. (1986). Secreted and surface antigens from larval stages of Wuchereria bancrofti, the major human lymphatic filarial parasite. Molecular and Biochemical Parasitology 15, 203-214.

Maizels, R.M., Denham, D.A. & Sutanto, I. (1985). Secreted and circulating antigens of the filarial parasite Brugia pahangi: analysis of in vitro released components and detection of parasite products in vivo. Molecular and Biochemical Parasitology 17, 277-288.

Maizels, R.M., Meghji, M. & Ogilvie, B.M. (1983). Restricted sets of parasite antigens from the surface of different stages and sexes of the nematode parasite Nippostrongylus brasiliensis. Immunology 48, 107-121.

Maizels, R.M., Partono, F., Oemijati, S., Denham, D.A. & Ogilvie, B.M. (1983). Cross reactive surface antigens on three stages of Brugia malayi, B.pahangi and B.timori. Parasitology 87, 249-263.

Maizels, R.M., Philipp, M. & Ogilvie, B.M. (1982). Molecules on the surface of parasitic nematodes as probes of the immune response in infection. Immunology Reviews 61, 109-136.

Markwell, M.A.K. & Fox, C.F. (1978). Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1,3,4,6-tetrachloro-3α-6α-diphenylglycouril. Biochemistry 17, 4807-4817.

May, R.M. & Anderson, R.M. (1978). Regulation and stability of host-parasite population interactions. II. Destabilizing processes. Journal of Animal Ecology 47, 249-268.

McL. Neilson, J.T. Forrester, D.J. & Thompson, N.P. (1973). Immunologic studies on Heligmosomoides polygyrus infection in the mouse: the dynamics of single and multiple infections and the effect of DDT upon acquired resistance. International Journal for Parasitology 3, 371-378.

Medley, G. & Anderson, R.M. (1985). Density-dependent fecundity in Schistosoma mansoni infections in man. Transactions of the Royal Society of Tropical Medicine and Hygiene 79, 532-534.

Meghji, M. & Maizels, R.M. (1986). Biochemical properties of larval excretory-secretory glycoproteins of the parasitic nematode Toxocara canis. Molecular and Biochemical Parasitology 18, 155-170.

Michel, J.F. (1963). The phenomena of host resistance and the course of infection of Ostertagia ostertagia in calves. Parasitology 53, 63-84.

Michel, J.F. (1970). The regulation of populations of Ostertagia ostertagia in calves. Parasitology 61, 435-447.

Miller, H.R.P. (1986). Vaccination against intestinal parasites. In: Parasitology-Quo Vadit? (ed.) M.J. Howell, pp. 43-51. Canberra.

Miller, H.R.P., Huntley, J.F. & Wallace, G.R. (1981). Immune exclusion and mucus trapping during the rapid expulsion of Nippostrongylus brasiliensis from primed rats. Immunology 44, 419-429.

Miller, H.R.P. & Jarrett, H.F.W. (1971). Immune reactions in mucuous membrane. I. Intestinal mast cell response during helminth expulsion in the rat. Immunology 20, 277-288.

Miller, T.A. (1971). Vaccination against the canine hookworm diseases. Advances in Parasitology 9, 153-183.

Miller, T.A. (1978). Industrial development and field use of the canine hookworm vaccine. Advances in Parasitology 16, 333-342.

Miller, T.A. (1979). Hookworm infection in man. Advances in in Parasitology 17, 315-365.

Mills, C.A., Anderson, R.M. & Whitfield, P.J. (1979). Density-dependent survival and reproduction within populations of the ectoparasitic digenean Transversotrema patialense on the fish host. Journal of Animal Ecology 48, 383-399.

Ministry of Agriculture, Fisheries and Food (MAFF)(1977). Manual of Veterinary Parasitological Laboratory Techniques. Technical Bulletin, 18.

Mitchell, A.L., Wescott, R.B. & Perryman, L.E. (1983). Kinetics of expulsion of the nematode, Nippostrongylus brasiliensis, in mast cell deficient w/w^{v} mice. Parasite Immunology 4, 1-12.

Mitchell, G.F. (1979). Responses to infection with metazoan and protozoan parasites in mice. Advances in Immunology 28, 451-511.

Mitchell, G.F., Anders, R.F., Brown, G.R., Handmann, E., Roberts-Thompson, I.O., Chapman, C.B., Forsyth, K.P., Kahe, L.P. & Cruise, K.M. (1982). Analysis of infection characteristics and anti-parasite immune responses in resistant compared with susceptible host. Immunological Reviews 61, 137-188.

Mitchell, G.F. & Cruise, K.M. (1984). Immunization with Nematospiroides dubius adult worms plus pertussigen has different consequences in mice of various genotypes. Australian Journal of Experimental Biology and Medical 62, 523-530.

Mitchell, G.F., Marchalonis, J.J., Smith, P.M., Nicholas, W.L. & Warner N.J. (1977). Studies on immune responses to larval cestodes in mice. Immunoglobulins associated with the larvae of Mesocestoides corti. Australian Journal of Experimental Biology and Medical Science 55, 187.

Mitchell, G.F. & Munoz, J.J. (1983). Vaccination of genetically susceptible mice against chronic infection with Nematospiroides dubius using pertussigen as adjuvant. Australian Journal of Experimental Biology and Medical Science 61, 425-434.

Mitchell, G.F. & Prowse, S.J. (1979). Three consequences of infection with Nematospiroides dubius in three inbred strains of mice. Journal of Parasitology 65, 820-822.

Molinari, J.A., Ebersole, J.L. & Cypess, R.H. (1978). Specific antibody levels in the serum of Heligmosomoides polygyrus-infected mice. Journal of Parasitology 64, 233-238.

Morgan, T.M., Sutanto, I., Partono, F., P., S., & Maizels, R.M. (1986). Antigenic characterization of adult Wuchereria bancrofti filarial nematodes. Molecular and Biochemical Parasitology (in press).

Muller, R. (1975). Worms and Disease. Heinemann, London.

Murua, R.E. (1978). Studies on the ecology of parasites of Apodemus sylvaticus (L.) and Clethrionomys glareolus (Schreb.)(Rodentia): analysis of the parasite populations and their seasonal variation in the Bristol area. Acta Parasitologica (Polonica) 25, 149-161.

Nash, D.R. & Heremans, J.F. (1969). A quantitative antibody-binding method for the determination of specific antibody within different immunoglobulin classes: application to four immunoglobulin classes in the mouse. Immunology 17, 685-694.

Nawa, Y. & Miller, H.R.P. (1979). Adoptive transfer of the intestinal mast cell response in rats infected with Nippostrongylus brasiliensis. Cellular Immunology 42, 225-239.

Newell, J. (1985). A vaccine for schistosomiasis. New Scientist 105, 23.

Ninneman, J.L. & Lueker, D.C. (1974). Mechanisms of murine immunity to Nematospiroides dubius after subcutaneous vaccination. Journal of Parasitology 60, 980-984.

Nwosu, A.B.C. (1981). Human neonatal infections with hookworms in an endemic area of Southern Nigeria. Tropical and Geographic Medicine 33, 105-111.

Nwosu, A.B.C. & Anya, A.O. (1980). Seasonality in hookworm infection in an endemic area of Nigeria, and its relationship to rainfall. Tropenmedizin und Parasitenkunde 31, 201-208.

Ogilvie, B.M., Bartlett, A., Godfrey, R.C., Turton, J.A. & Yeates, R.A. (1978). Antibody responses in self infections with Necator americanus. Transactions of the Royal Society of Tropical Medicine and Hygiene 63, 363-369.

Ogilvie, B.M. & De Savigny, D. (1982). Immune response to nematodes. In: Immunology of Parasitic Infections, (eds.) S. Cohen & K.S. Warren, pp. 716-757. Blackwell Scientific Publications, Oxford.

Ogilvie, B.M. & Love, R.J. (1974). Co-operation between antibodies and cells in immunity to a nematode parasite. Transplantation Reviews 19, 147-168.

Ogilvie, B.M., Philipp, M., Jungery, M., Maizels, R.M., Worms, M.J. & Parkhouse, R.M.E. (1980). The surface of nematodes and immune response of the host. In: The Host-Invader Interplay, (ed.) H. van den Bossche, Elsevier Amsterdam.

Ouiassi, A., Kouemeni, L.-E., Haque, A., Ridel, P.-R, Andre, P.S. & Capron, A. (1981). Detection of circulating antigens in onchocerciasis. American Journal of Tropical Medicine and Hygiene 30, 1211-1218.

Palmas, C., Bortoletti, G., Conchedda, M. & Gabriele, F. (1986). Immunological memory and lymphoblast-migration in mice infected with Hymenolepis nana. Zeitschrift fur Parasitenkunde 72, 397-403.

Panter, H.C. (1969). The mechanism of immunity of mice to Nematospiroides dubius. Journal of Parasitology 55, 38-43.

Papasurathorn, T., Chularerk, P., Chearanai, S., Chularerk, U., Kiarttivuti, B., Viboolyanvatua Krrttivuti, A. & Vongtanswad, S. (1967). Study on ecology and prevalence of intestinal parasites with special reference to the intensity of human hookworm infections and opisthorchiasis in health development area, Bantard Udoin Province. Journal of the Medical Association of Thailand 50, 423-445.

Parker, S.J. & Inchley, C.J. (1985). FACS analysis of lymphoid tissues in mice infected with Nematospiroides dubius. Transactions of the Royal Society of Tropical Medicine and Hygiene 79, 869.

Parkhouse, R.M.E., Clark, N.W.T., Maizels, R.M. & Denham, D.A. (1985). Brugia pahangi: labelling of secreted antigens with ^{35}S-methionine in vitro. Parasite Immunology 7, 665-668.

Parkhouse, R.M.E., Philipp, M. & Ogilvie, B.M. (1981). Characterization of surface antigens of Trichinella spiralis infective larvae. Parasite Immunology 3, 339-352.

Pentilla, I.A., Ey, P.L. & Jenkin, C.R. (1983). Adherence of murine peripheral blood eosinophils and neutrophils to the different parasitic stages of Nematospiroides dubius. Australian Journal of Experimental Biology and Medical Science 61, 617-627.

Pentilla, I.A., Ey, P.L. & Jenkin, C.R. (1984a). Reduced infectivity of Nematospiroides dubius larvae after incubation in vitro with neutrophils or eosinophils from infected mice and a lack of effect by neutrophils from normal mice. Parasite Immunology 6, 295-308.

Pentilla, I.A., Ey, P.L. & Jenkin, C.R. (1984b). Infection of mice with Nematospiroides dubius: demonstration of neutrophil-mediated immunity in vivo in the presence of antibodies. Immunology 53, 147-154.

Pentilla, I.A., Ey, P.L., Lopez, A.F. & Jenkin, C.R. (1985). Suppression of early immunity to Nematospiroides dubius in mice by selective depletion of neutrophils with monoclonal antibody. Australian Journal of Experimental Biology and Medical Science 63, 531-543.

Pesigan, T.P., Farooq, M., Hairston, N.G., Jauregui, J.J., Garcia, E.G., Santos, A.T., Santos, B.C. & Besa, A.A. (1958). Studies on Schistosoma japonicum infection in the Philippines. I. General considerations and epidemiology. Bulletin of the World Health Organisation 18, 345-455.

Peters, W. (1978). Medical aspects: comments and discussion. II. In: The Relevance of Parasitology to Human Welfare Today, (eds.) A.E. Taylor & R. Muller, Symposium of the British Society for Parasitology, vol. 16. Blackwell Scientific Publications, Oxford.

Philipp, M., Gomez-Priego, A., Parkhouse, R.M.E., Davies, M.W., Clark, N.W.T., Ogilvie, B.M. & Beltran-Hernandez, F. (1984). Identification of an antigen of Onchocerca volvulus of possible diagnostic use. Parasitology 89, 295-309.

Philipp, M., Maizels, R.M., McLaren, D.J., Davies, M.W., Suswillo, R. & Denham, D.A. (1986). Expression of cross-reactive surface antigens by different stages of Brugia pahangi during infections in cats. Transactions of the Royal Society of Tropical Medicine and Hygiene (in press).

Philipp, M., Parkhouse, R.M.E. & Ogilvie, B.M. (1980). Changing proteins on the surface of a parasitic nematode. Nature 287, 538-540.

Philipp, M. & Rumjanek, F.D. (1984). Antigenic and dynamic properties of helminth surface structures. Molecular and Biochemical Parasitology 10, 245-268.

Philipp, M., Taylor, P.M., Parkhouse, R.M.E. & Ogilvie, B.M. (1981). Immune response to stage-specific surface antigens of the parasitic nematode Trichinella spiralis. Journal of Experimental Medicine 154, 210-215.

Pielou, E.C. (1981). The usefulness of ecological models: a stock-taking. Quarterly Review of Biology 56, 17-31.

Pradhan, S.L. & Johnstone I.L. (1972). Haemonchus contortus- the effect on lambs of prolonged exposure to daily and weekly doses of infective larvae. Parasitology 64, 143-152.

Price, P.W. (1980). Evolutionary Biology of Parasites. Princeton University Press, Princeton.

Pritchard, D.I. & Behnke, J.M. (1985). The suppression of homologous immunity by soluble adult antigens of Nematospiroides dubius. Journal of Helminthology 59, 251-256.

Pritchard, D.I., Ali, N.M.H. & Behnke, J.M. (1984). Analysis of the mechanism of immunodepression following heterologous antigenic stimulation during concurrent infection with Nematospiroides dubius. Immunology 51, 633-642.

Pritchard, D.I., Williams, D.J.L., Behnke, J.M. & Lee, T.D.G. (1983). The role of IgG1 hypergammaglobulinaemia in immunity to the gastrointestinal nematode Nematospiroides dubius. The immunochemical purification, antigen specificity and in vivo anti-parasite effect of IgG1 from immune serum. Immunology 49, 353-365.

Pritchard, D.I., Behnke, J.M. & Williams, D.J.L. (1984). Primary infection sera and IgG1 do not block host-protective immunity to Nematospiroides dubius. Immunology 51, 73-81.

Pritchard, D.I., Crawford, C.R., Duce, I.R. & Behnke, J.M. (1985). Antigen stripping from the nematode epicuticle using the cationic detergent cetyl trimethyl-ammonium bromide (CTAB). Parasite Immunology 7, 575-585.

Pritchard, D.I., Maizels, R.M., Behnke, J.M. & Appleby, P. (1984). Stage-specific antigens of Nematospiroides dubius. Immunology 53, 325-335.

Prowse, S.J. (1982). Interleukin 2 secretion by sensitized cells from mice infected with Nematospiroides dubius: kinetics and specificity of the response. Parasite Immunology 4, 363-372.

Prowse, S.J., Ey, P.L. & Jenkin, C.R. (1978). Immunity to Nematospiroides dubius: cell and immunoglobulin changes associated with the onset of immunity in mice. Australian Journal of Experimental Biology and Medical Science 56, 237-246.

Prowse, S.J., Ey, P.L. & Jenkin, C.R. (1979). Alternative pathway activation of complement by a murine parasitic nematode (Nematospiroides dubius). Australian Journal of Experimental Biology and Medical Science 57, 459-466.

Prowse, S.J. & Mitchell, G.F. (1980). On the choice of mice for dissection of strain variations in the development of resistance to infection with Nematospiroides dubius. Australian Journal of Experimental Biology and Medical Science 58, 603-605.

Prowse, S.J., Mitchell, G.F., Ey, P.L. & Jenkin, C.R. (1978). Nematospiroides dubius: susceptibility to infection and the development of resistance in hypothymic (nude) BALB/c mice. Australian Journal of Experimental Biology and Medical Science 56, 561-570.

Prowse, S.J., Mitchell, G.F., Ey, P.L. & Jenkin, C.R. (1979). The development of resistance in different inbred strains of mice to infection with Nematospiroides dubius. Parasite Immunology 1, 277-288.

Rainbow, V.M.T. (1972). Studies on the helminth parasites of small mammals with particular reference to the ecology and physiology of Nematospiroides dubius. PhD Thesis, University of London.

Rau, M.E. (1983a). The open-field behaviour of mice infected with Trichinella spiralis. Parasitology 86, 311-318.

Rau, M.E. (1983b). Establishment and maintenance of behavioural dominance in male mice infected with Trichinella spiralis. Parasitology 86, 319-322.

Rau, M.E. (1984). Loss of behavioural dominance in male mice infected with Trichinella spiralis. Parasitology 88, 371-373.

Rausch, R. (1952). Studies on the helminth fauna of Alaska. X1. Helminth parasites of microtine rodents - taxonomic considerations. Journal of Parasitology 38, 415-444.

Reimer, J.D. & Petras, M.L. (1967). Breeding structure of the house mouse, Mus musculus, in a population cage. Journal of Mammalogy 31, 403-410.

Rice, M.C. & O'Brien, S.J. (1980). Genetic variance of laboratory outbred Swiss mice. Nature 283, 157-161.

Roche, M. & Layrisse, M. (1966). The nature and causes of 'hookworm anaemia'. American Journal of Tropical Medicine and Hygiene 15, 1031-1102.

Rogers, W.P. (1986). Advances in parasitology: 1886-1986. In: Parasitology-Quo Vadit? (ed.) M.J. Howell, pp. 1-13. Canberra.

Roitt, I., Brostoff, J. & Male, D. (1985). Immunology. Churchill Livingstone, Edinburgh.

Roman, E. (1951). Etude ecologique et morphologique sur les Acanthocephales et les nematodes parasites des rats de la region Lyonnaise. Memoires de Museum de Nationale d'Histoire Naturelle, Series A. Zoologie 2, 249-270.

Rubin, R., Lueker, D.C., Flom, J.L. & Andersen, S. (1971). Immunity against Nematospiroides dubius in CFW Swiss-Webster mice protected by subcutanéous larval vaccination. Journal of Parasitology 57, 815-817.

Ruitenberg, E.J. & Elgersma, A. (1976). Absence of intestinal mast cell response in congenitally athymic mice during Trichinella spiralis infection. Nature 264, 258-260.

Sayles, P.C. & Jacobson, R.H. (1983). Effects of various anthelmintics on larval stages of Nematospiroides dubius (Nematoda). Journal of Parasitology 69, 1079-1083.

Schad, G.A. & Anderson, R.M. (1985). Predisposition to hookworm infection in humans. Science 228, 1537-1540.

Schad, G.A. & Banwell, J.G. (1984). Hookworms. In: Tropical and Geographical Medicine, (eds.) K.S. Warren & A.A.F. Mahmoud, pp. 358-372. McGraw-Hill, New York.

Schad, G.A., Nawalinski, T.A. & Kochar, V. (1983). Human ecology and the distribution and abundance of hookworm populations. In: Human Ecology and Infectious Diseases, (eds.) N.A. Croll & J. Cross, pp. 187-223. Academic Press, New York.

Schmitz, B., Possart-Schmitz, P., Gehrung, M., Stauffer, U., Mossmann, H. & Fischer, H. (1982). Cellular response and resistance to the primary infection of rats and mice with Nematospiroides dubius. Zeitschrift fur Parasitenkunde 68, 339-347.

Scott, M.E. (1985). Experimental epidemiology of Gyrodactylus ballatarudis (Monogenea) on guppies (Poecilia reticulata): short- and long-term studies. In: Ecology and Genetics of Host-Parasite Interactions, (eds.) D. Rollinson & R.M. Anderson, pp. 21-38. Academic Press, London.

Scott, M.E. (1986). Suppression of mouse colony abundance by a helminth parasite infection.(In preparation).

Sedmak, J.J. & Grossberg, S.E. (1977). A rapid, sensitive, and versatile assay for protein using Coomassie Brilliant Blue G250. Analytical Biochemistry 79, 544-552.

Selkirk, M.E., Denham, D.A., Partono, F., Sutanto, I. & Maizels, R.M. (1986). Molecular characterization of antigens of lymphatic filarial parasites. Parasitology 91, S15-S38.

Sen, H.G. & Seth, D. (1967). Complete development of the human hookworm Necator americanus in golden hamsters, Mesocricetus auratus. Nature 214, 609-610.

Sharpe, G.I. (1964). The helminth parasites of some small mammal communities. I. The parasites and their hosts. Parasitology 54, 145-154.

Shimp, R.G., Crandall, R.B. & Crandall, C.A. (1975). Heligmosomoides polygyrus: suppression of antibody response to orally adminstered sheep erythrocytes in infected mice. Experimental Parasitology 38, 257-269.

Simpson, A.J.G. (1986). The influence of molecular heterogeneity in helminth identification, protective immunity and immunodiagnosis. In: Parasitology-Quo Vadit? (ed.) M.J. Howell, pp. 69-77. Canberra.

Sitepu, P. & Dobson, C. (1982). Genetic contro of resistance to infection with Nematospiroides dubius in mice: selection of high- and low-immune response populations. Parasitology 85, 73-84.

Sitepu, P., Dobson, C. & Brindley, P.J. (1984). Infections with Salmonella typhimurium and Nippostrongylus brasiliensis in mice selectively reared for high or low immune responsiveness to Nematospiroides dubius. Australian Journal of Experimental Biology and Medical Science 62, 755-761.

Sitepu, P., Dobson, C. & Brindley, P.J. (1985). Immunization and immunosuppression in mice reared for high or low immune responsiveness against Nematospiroides dubius. International Journal for Parasitology 15, 277-281.

Skamene, E., Kongshavn, P.A.L. & Landy, M. (eds.) (1980). Genetic Control of Natural Resistance to Infection and Malignancy. Academic Press, New York.

Skamene, E., Gros, P., Forget, A., Kongshavn, P.A.L., St. Charles, C. & Taylor, B.A. (1982). Genetic regulation of resistance to intracellular pathogens. Nature 297, 506-509.

Slater, A.F.G. & Keymer, A.E. (1986a). Epidemiology of Heligmosomoides polygyrus in mice: experiments on natural transmission. Parasitology 93, 177-188.

Slater, A.F.G. & Keymer, A.E. (1986b). Heligmosomoides polygyrus (Nematoda): the influence of dietary protein on the dynamics of repeated infection. Proceedings of the Royal Society of London (in press).

Smal, C.M. & Fairley, J.S. (1982). The dynamics and regulation of small rodent populations in the woodland ecosystems of Killarney, Ireland. Journal of Zoology (London) 196, 1-30.

Smith, G. & Grenfell, B. (1984). The influence of water temperature and pH on the survival of Fasciola hepatica miracidia. Parasitology 88, 97-104.

Smith, G. & Grenfell, B. (1985). Population dynamics of Ostertagia ostertagia. Parasitology Today,1 , 76-81.

Smithers, S.R. (1986). Vaccination against schistosomes and other systemic helminths. In: Parasitology-Quo Vadit? (ed.) M.J. Howell, pp. 31-42. Canberra.

Smithers, S.R. & Terry, R.J. (1965). The infection of laboratory hosts with cercariae of Schistosoma mansoni and recovery of adult worms. Parasitology 55, 695-700.

Sokal, R.R. & Rohlf, F.J. (1981). Biometry. Freeman, San Fransisco.

Soller, M. & Genizi, A. (1967). Optimum experimental designs for realized heritability estimates. Biometrics 23, 361-365.

Southwood, T.R.E. (1978). Ecological Methods. Chapman and Hall, London.

Spurlock, G.M. (1943). Observations on host-parasite relations between laboratory mice and Nematospiroides dubius Baylis. Journal of Parasitology 29, 303-311.

Stephenson, L.S., Latham, M.C., Kurz, K.M., Kinoti, S.N., Oduori, M.L. & Crompton, D.W.T. (1985). Relationships of Schistosoma haematobium, hookworm and malarial infections and metrifonate treatment to growth of Kenyan school children. American Journal of Tropical of Tropical Medicine and Hygiene 34, 1109-1118.

Stevens, D.P. (1978). Quantitative techniques. In: Clinics in Gastroenterology 7, 231-238.

Stoll, N.R. (1947). This wormy world. Journal of Parasitology 33, 1-17.

Sutanto, I., Maizels, R.M. & Denham, D.A. (1985). Surface antigens of a filarial nematode: analysis of adult Brugia pahangi surface components and their use in monoclonal antibody production. Molecular and Biochemical Parasitology 15, 203-214.

Sukhdeo, M.V.K.& Croll, N.A. (1981). The location of parasites within their hosts: bile and the site of selection behaviour of Nematospiroides dubius. International Journal for Parasitology 11, 157-162.

Sukhdeo, M.V.K. & Mettrick, D.F. (1983). Site selection by Heligmosomoides polygyrus (Nematoda): effects of surgical alteration of the gastrointestinal tract. International Journal for Parasitology 13, 355-358.

Svejgaard, A. (1980). HLA and disease. In: Manual of Clinical Immunology, (eds.) N.R. Rose & H. Friedman, American Society for Microbiology, Washington.

Sweet, W.C. (1925). Hookworm re-infections. Ceylon Journal of Medical Science 1, 129-140.

Taylor, L.R. (1961). Aggregation, variance and the mean. Nature 189, 732-735.

Thomas, R.J. (1953). On the nematode and trematode parasites of some small mammals from the Inner Hebrides. Journal of Helminthology 27, 143-168.

Uber, C.L., Roth, R.L. & Levy, D.A. (1980). Expulsion of Nippostrongylus brasiliensis by mice deficient in mast cells. Nature 287, 226-228.

Udonsi, J.K., Nwosu, A.B.C. & Anya, A.O. (1980). Necator americanus: population structure, distribution, and flactuations in population densities of infective larvae in contaminated farm lands. Zeitschrift fur Parasitenkunde 63, 257-259.

Udonsi, J.K. (1983). Necator americanus infection: a longitudinal study of an urban area in Nigeria. Annals of Tropical Medicine and Parasitology 77, 305-310.

Van den Bossche, H. (1978). Chemotherapy of parasitic infections. Nature 273, 626-630.

Van Zandt, P.D. (1961). Studies on the immunity relationship in white mice given infections with Nematospiroides dubius. Journal of the Elisha Mitchell Scientific Society 77, 300-309.

Van Zandt, P.D. (1971). Immunity relationships in white mice infected with Nematospiroides dubius. III. Artificial immunization with antigen prepared from adults. Journal of the Elisha Mitchell Scientific Society 87, 74-76.

Van Zandt, P.D., Cypess, R.H. & Zidian, J.L. (1973). Development of age and sex resistance to Nematospiroides dubius in the mouse following single and multiple infections. Journal of Parasitology 59, 977-979.

Vaupel, J.W. & Yashin, A.I. (1985). Heterogeneity's ruses: some surprising effects of selection on population dynamics. The American Statistician 39, 176-185.

Voller, A., Bidwell, D. & Bartlett, A. (1976). The application of micro-plate enzyme-linked immunosorbent assays to some infectious diseases. In: Ist International Symposium on Immunoenzymatic Techniques INSERM, (eds.) Feldman et al., pp, 167-173.

Voller, A., Bidwell, D. & Bartlett, A. (1980). Enzyme-linked immunosorbent assay. In: Manual of Clinical Immunology, (eds.) N.R. Rose & H. Friedman, pp. 359-371. American Society for Microbiology, Washington.

Voller, A. & De Savigny, D. (1981). Diagnostic serology of tropical parasitic diseases. Journal of Immunologic Methods 46, 1.

Wakelin, D. (1973). The stimulation of immunity to Trichuris muris in mice exposed to low-level infections. Parasitology 66, 181-189.

Wakelin, D. (1975a). Genetic control of immune responses to parasites: selection for responsiveness and non-responsiveness to Trichuris muris in random-bred mice. Parasitology 71, 377-384.

Wakelin, D. (1975b). Immune expulsion of Trichuris muris from mice during a primary infection: analysis of the components involved. Parasitology 70, 397-405.

Wakelin, D. (1978a). Immunity to intestinal parasites. Nature 273, 617-620.

Wakelin, D. (1978b). Genetic control of susceptibility and resistance to parasitic infection. Advances in Parasitology 16, 219-308.

Wakelin, D. (1980). Genetic control of immunity to parasites. Infection with Trichinella spiralis in inbred and congenic mice showing rapid and slow responses to infection. Parasite Immunology 2, 85-98.

Wakelin, D. (1982). Mouse models of genetic variation in resistance to helminth parasites. In: Animal Models in Parasitology, (ed.) D.G. Owen, ppl 53-68. MacMillan, London.

Wakelin, D. (1984a). Immunity to Parasites. Edward Arnold, London.

Wakelin, D. (1984b). Evasion of the immune response: survival within low responder individuals of the host population. Parasitology 88, 639-657.

Wakelin, D. (1985a). Genetic control of immunity to helminth infections. Parasitology Today 1, 17-23.

Wakelin, D. (1985b). Genetics, immunity and parasite survival. In: Ecology and Genetics of Host-Parasite Interactions, (eds.) D. Rollinson & R.M. Anderson, pp. 39-54. Academic Press, London.

Wakelin, D., Grencis, R.K. & Donachie, A.M. (1982). Short-lived, dividing cells mediate adoptive transfer of immunity to Trichinella spiralis in mice. II. In vivo characteristics of the cells. Immunology 46, 451-457.

Wakelin, D. & Lloyd, M. (1976). Accelerated expulsion of adult Trichinella spiralis in mice given lymphoid cells and serum from infected donors. Parasitology 72, 173-182.

Walton, A.C. (1923). Some new and little-known nematodes. Journal of Parasitology 2, 59-70.

Wang, C.C. (1982). Current problems in anti-parasite chemotherapy. Trends in Biochemical Science 7, 354-356.

Warren, K.S. (1973). Regulation of the prevalence and intensity of schistosomiasis in man: immunology or ecology? Journal of Infectious Diseases 127, 595-607.

Warren, K.S. (1981). The control of helminths: non-replicating infectious agents of man. Annual Review of Public Health 2, 101-115.

Warren, K.S. & Mahmoud, A.A.F. (1976). Targetted mass treatment: a new approach to the control of schistosomiasis. Transactions of the Association of American Physicians 89, 195-204.

Warren, K.S. & Mahmoud, A.A.F. (1984).(eds.). Tropical and Geographic Medicine. McGraw-Hill, New York.

Wassom, D.L., DeWitt, C.W. & Grundmann, A.W. (1974). Immunity to Hymenolepis citelli by Peromyscus maniculatus: genetic control and ecological implications. Journal of Parasitology 60, 47-52.

Wassom, D.L., David, C.S. & Gleich, G.J. (1979). Genes within the major histocompatibility complex influence susceptibility to Trichinella spiralis in the mouse. Immunogenetics 9, 491-496.

Wassom, D.L., Wakelin, D., Brooks, B.O., Krco, C.J. & David, C.S. (1984). Genetic control of immunity to Trichinella spiralis infections in mice. Hypothesis to explain the role of H-2 genes in primary and challenge infections. Immunology 51, 625-631.

Wassom, D.L., Dick, T.A., Arnason, N., Strickland, D. & Grundmann, A.W. (1986). Host genetics: a key factor in regulating the distribution of parasites in natural host populations. Journal of Parasitology 72, 334-337.

Wenlock, R.W. (1978). Age variation in prevalence of parasitic diseases in rural communities. Medical Journal of Zambia 12, 13-15.

Williams, D.J. & Behnke, J.M. (1983). Host protective antibodies and serum immunoglobulin isotypes in mice chronically infected or repeatedly immunized with the nematode parasite Nematospiroides dubius. Immunology 48, 37-47.

Wilson, E.O. (1975). Sociobiology. Harvard University Press, Cambridge.

Wolton, R.J. & Flowerdew, J.R. (1985). Spatial distribution and movements of wood mice, yellow-necked mice, and bank voles. In: The Ecology of Woodland Rodents, Bank Voles and Wood Mice, (eds.) J.R. Flowerdew, J. Gurnell & J.H.W. Gripps, pp. 249-269. Clarendon Press, Oxford.

World Health Organisation (W.H.O.)(1981). Intestinal protozoal and helminthic infections. W.H.O. Technical Report Series No. 666.

Yamaguti, S. (1961). Systema Helminthum. Vol. III. The Nematodes of Verterbrates. Parts 1 & 2. Interscience Publishers, New York.

Yasuraoka, K. & Weinstein, P.P. (1969). Effects of temperature on the development of eggs of Nematospiroides dubius under axenic conditions relative to in vitro cultivation. Journal of Parasitology 55, 44-50.

Zar, J. (1984). Biostatistical Analysis. Prentice Hall, New Jersey.

APPENDIX

A.5.1 Trickle infections in low responder CBA/Ca mice

Rate of infection (/wk/mouse)	Weeks after first infection	number of mice	MWB±S.E.	VMR	Eggs/16h /mouse/♀ worm±S.E.
10L3	2	10	5.7±1.1	2.7	575±248
	4	10	13.9±1.9	2.8	853±150
	6	10	25.7±3.1	3.7	1005± 86
	8	10	17.9±4.9	13,9	649±169
	10	10	14.8±3.7	9.4	305±121
	12	10	7.3±3.1	13.2	233±111
25L3	2	10	22.6±2.2	2.9	607±117
	4	10	40.8±4.6	5.1	1075±119
	6	10	58.3±8.9	13.5	939± 76
	8	10	35.1±3.7	3.9	1231±144
	10	10	26.0±8.6	28.7	632±130
	12	10	30.7±9.2	27.5	755±134
50L3	2	10	34.8±3.8	4.1	656± 62
	4	10	77.0±6.4	7.5	738± 65
	6	10	87.3±11.1	14.1	774±110
	8	10	101.2±19.9	39.3	639±107
	10	8/10	143.4±21.8	26.6	998± 89
	12	7/10	150.6±16.2	12.1	796± 82

A.5.2 Trickle infections in high responder NIH mice

Rate of infection (/wk/mouse)	Weeks after first infection	number of mice	MWB±S.E.	VMR	Eggs/16h/ mouse/♀ worm ±S.E.
10L3	2	8	3.7±0.8	1.4	84.7± 32
	4	8	5.6±2.0	5.7	8.5± .8
	6	8	3.9±1.4	4.2	720.3±475
	8	8	1.4±0.6	2.1	0
	10	8	2.6±1.7	8.8	0
	12	10	0.1±0.1	1.0	0
25L3	2	8	11.7±1.7	1.9	123.9± 43
	4	8	6.1±1.5	3.0	11.5± 9
	6	8	4.9±1.6	4.4	25.7± 26
	8	8	1.2±0.5	1.5	0
	10	8	0.9±0.6	3.4	0
	12	10	0.1±0.1	1.0	0

A.5.2 (continued)

Rate of infection (/wk/mouse)	Weeks after first infection	number of mice	MWB±S.E.	VMR	Eggs/16h/ mouse/ worm ±S.E.
50L3	2	8	32.9±9.9	23.9	151.2± 23
	4	8	13.9±7.9	36.4	30.1± 28
	6	8	9.4±3.9	13.0	24.3± 21
	8	8	2.5±0.5	0.7	0
	10	8	1.0±0.6	2.6	0
	12	10	0.2±0.1	0.9	0

MWB = mean worm burden
VMR = variance to mean ratio

B.5.1 Trickle infections in CBA/Ca mice with chemotherapy*
(chemotherapy after 3 weeks of initial trickle infection)

Rate of infection (/wk/mouse)	Weeks after first infection	number of mice	MWB±S.E.	Eggs/16h/female worm ±S.E.
10L3	0	-	0	0
	3	10	12.8±2.1	853±150
	4	-	0	0
	6	10	4.8±1.2	347±129
	8	10	12.0±1.4	1186± 93
	10	10	7.8±2.3	843±176
	12	10	8.8±1.6	258± 88
	14	10	6.8±2.2	4± 3
50L3	0	-	0	0
	3	10	39.4±13.3	738± 65
	4	-	0	0
	6	10	20.4±2.8	476± 77
	8	10	23.5±4.6	925±141
	10	10	12.6±2.6	387±100
	12	10	10.3±2.9	77± 42
	14	10	20.9±12.5	45± 31

* 10 mg/kg body weight

B.5.2 Trickle infections in CBA/Ca mice with chemotherapy (chemotherapy after 6 weeks initial trickle infection)

Rate of infection (/wk/mouse)	Weeks after first infection	number of mice	MWB±S.E.	Eggs/16h/female worm ±S.E.
50L3	0		0	0
	2	10	34.8±3.8	656± 62
	3	10	39.4±13.3	
	4	10	77.0±6.4	738± 65
	6	10	112.2±9.7	774±110
	7		0	0
	8	10	14.7±1.9	164± 85
	10	10	11.0±2.5	278± 91
	12	10	19.1±11.1	117± 54
	14	10	6.1±1.8	57± 39
	16	10	9.6±2.8	48± 28
10L3	0		0	0
	2	10	5.7±1.1	575±248
	3	10	12.8±2.1	
	4	10	13.9±1.9	853±150
	6	10	27.4±3.7	1005± 86
	7		0	0
	8	10	2.6±0.4	10± 10
	10	10	5.6±1.3	556±115
	12	10	4.9±1.6	128± 43
	14	10	1.4±0.5	0
	16	10	2.2±0.5	130± 87

C.5.1 Long-term duration of acquired resistance in CBA/Ca mice during repeated infection with H.polygyrus

Interval between initial and second round of trickle infection after chemotherapy/ vaccination	Mean worm burdens ± S.E. (n) Control	Experimental
1 week	77.0± 6.4(10)	23.5± 4.6(10)
3 weeks	62.6± 6.2(5)	1.6± 0.6(5)
5 weeks	60.0± 9.2(5)	7.2± 4.7(5)
7 weeks	61.8± 4.9(5)	1.2± 0.4(5)
7 weeks (vaccination)	61.8± 4.9(5)	4.5± 0.6(5)

n = number of mice

C.5.2 Vaccination of high (NIH) and low (CBA/Ca) responder mice with 250 irradiated H.polygyrus larvae

	Mean worm burdens ± S.E. (n) Control	Experimental
High responders	9.4±3.9(8)	0.5±0.3 (11)
Low responders	87.3±11.1(10)	34.8±13.0 (10)